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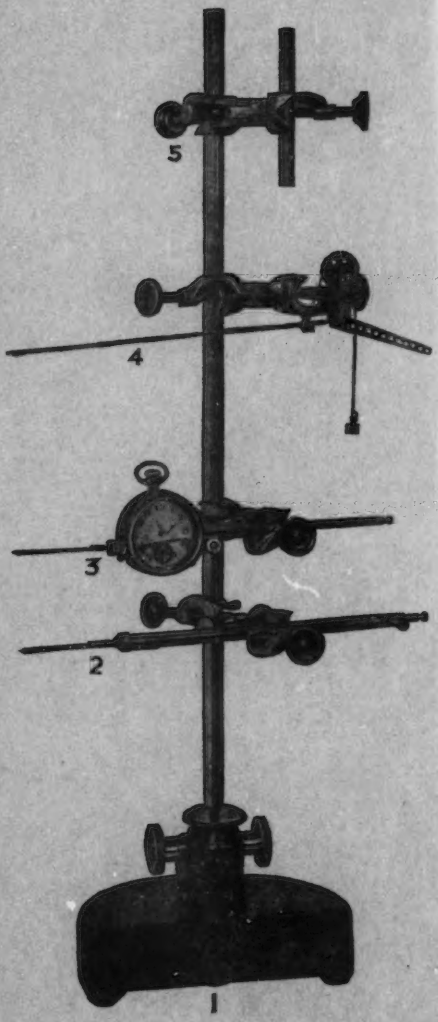

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WEIGHT AND THE MONTH OF BIRTH

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I.

In April, 1926, it was observed that the median weights of Boston School children of precisely the same age varied according to the time of the year in which these children were born. Thus boys 109 months of age born in February, March and April were lighter than boys 109 months of age born in October, November and December. The former weighed 57.2 pounds, while the latter weighed 61.3 pounds. At 121 months of age the February, March and April boys weighed 62.3 pounds and the October, November and December boys weighed 66.0 pounds, and at 134 months of age the respective weights were 69.3 and 72.2 pounds. Still another example may be given. The combined median weight of boys 159 and 160 months old was 85.0 pounds for those born between January 1 and June 30, but it was 88.6 pounds for those of the same age (159 and 160 months) born between July 1 and December 31. Those born in the second half of the year had in this case an advantage of several pounds.

On the other hand, there were ages in which boys born in the second half of the year had no advantage over those born in the first half. Thus at months 143 and 144 the combined median weight was 75.6 pounds for boys born between January 1 and June 30, and it was 75.7 pounds for boys of the same age (143 and 144 months) born between July 1 and December 31. Similarly, at 155 and 156 months of age the January-June boys weighed 81.9 pounds, while the July-December boys weighed 81.7 pounds.

Finally, there were ages in which boys born in the first half of the year were heavier than those of equal age born in the second half of the year. Thus the median weights of boys 152, 153, 154 and 155 months old, averaged, was 1.2 pounds greater for boys born January to June than for boys

[1] Mr. Baird, a student in the Harvard Medical School, worked in this research from July to September, 1926. His able assistance is gratefully acknowledged.

born July to December. In the case of girls at these months the difference was 1.8 pounds in favor of those born in the first half of the year.

In short, the median weights of school children born in the first half of the year sometimes rose above, sometimes equalled, and sometimes fell below the median weights of children born in the second half of the year, although within each of these three groups the January-June children and the July-December children were respectively of equal age.

II.

It is easy to understand these surprising data by studying figure 1, which may be regarded as a diagram to illustrate seasonal growth, as demonstrated in this journal in 1920.[2] It appears from figure 1 that January-

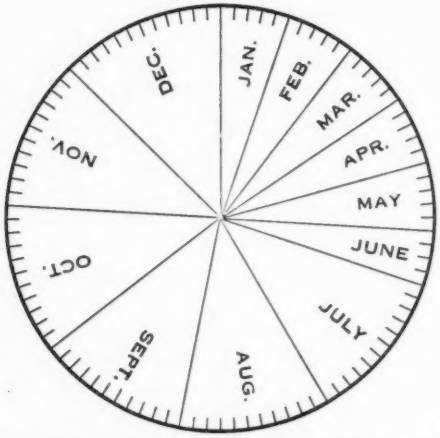

Fig. 1. A diagram to illustrate the months of slow and of rapid growth. The periphery of the circle is divided into ounces.

June are months of slow growth, whereas July-December are months of rapid growth. Let us now consider the growth of two groups of boys, A and B, each of the same age, for example, 144 months. Since 144 months is 12 full years of 12 months each, it follows that group A and group B will each have passed 12 times through the 6 months of slow growth and 12 times through the 6 months of rapid growth. Each group will in all have had 72 months of slow and 72 months of rapid growth. Neither group will have an advantage over the other. But this equality, just demonstrated for 144 months, must also hold at 156 months, which con-

[2] Porter, W. T. The seasonal variation in the growth of Boston School Children. This Journal, 1920, lii, 121.

tains 13 full periods of 12 months each, and indeed it will be true for any number of months exactly divisible by 12. Moreover, it will be true without regard to the month in which these boys were born. The boys of group A may all have been born January 1, at the beginning of the period of slow growth, and the boys of group B may have been born July 1, at the beginning of the period of rapid growth, but at 72, 84, 96, 108, 120, 132, 144, and 156 months[3] of age, each group will in every space of 12 months have passed equally through the slow and the fast period of growth.

Let us take now a further step. Group A and group B will now be of an age in which the number of months is no longer divisible by 12. Let them be 148 months old. Since up to 144 months each group had an equal number of slow and fast periods, we need concern ourselves only with the 4 months in excess of 144. Let us suppose the boys of group A

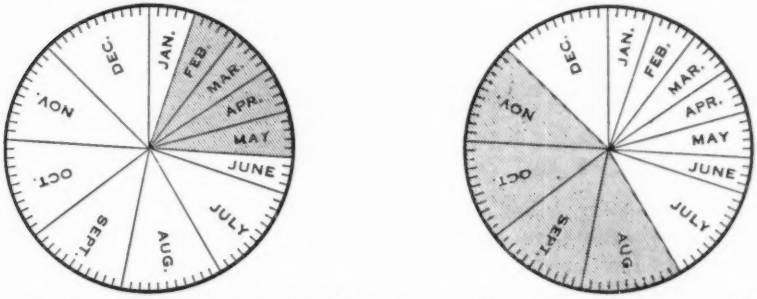

Fig. 2. A diagram to show the difference in growth between two boys each 12 years and 4 months old. One boy, born February first, has his 4 months in a period of slow growth. The other boy, born August first, has his 4 months in a period of rapid growth.

to have been born February first and those of group B to have been born August first. Then, as in figure 2, the February boys will have their 4 months in a period of slow growth and the August boys will have their 4 months in a period of rapid growth. In the 148 months of their age, the August boys will have had 4 more months of rapid growth than the February boys have had. Hence the average weight of the August boys will be greater than the weight of the February boys though both groups are of the same age, 148 months.

At 155 months the August boys will have lost their advantage. Eleven months will have passed since the last period of equality, at 144 months. For the August boys, only the first 5 months of these eleven will be months of rapid growth; but the February boys, after passing through 5 months

[3] The years here cited are those studied in the present investigation.

of slow growth, will have 6 months of rapid growth, at 155 months, therefore, the February group will average heavier than the group born in August.

When similar calculations are made for other months of birth and for other months of age, table 1 will be the result (see page 5). In table 1 the months of rapid growth throughout the yearly age cycle are set down opposite the birth months from January to December.

III.

It will be interesting to test by actual observations the validity of the propositions laid down in section II.

It has been stated that in months of age divisible by 12, the weight of children born at one time of the year should equal that of children born at another time of the year. At 144 months of age the median weight of boys born in the four quarters of the year was as follows:

	pounds
First quarter	75.8
Second quarter	75.0
Third quarter	75.8
Fourth quarter	75.8

At 149 months, July-December boys should be heavier than January-June boys, since the former have more months of rapid growth. The observed figures are 79.7 and 77.7 pounds.

At 153 months, January-June girls should be heavier than July-December girls since the former have 6 more months of rapid growth. The observed weights are 83.6 and 81.3 pounds.

We learn from table 1 that in months 145, 146, 147, 148, 149 and 150 the July-December children should be heavier than those born in January-June. In months 152, 153, 154 and 155, the reverse should be expected. Finally, in months 157, 158, 159 and 160, the July-December children should again be heavier. The actual observations follow:

MONTHS OF AGE	JANUARY-JUNE BOYS	JULY-DECEMBER BOYS	JANUARY-JUNE GIRLS	JULY-DECEMBER GIRLS
	pounds	*pounds*	*pounds*	*pounds*
145–150	77.2	78.6	77.6	78.6
152–155	81.2	80.5	84.1	82.3
157–160	84.0	87.6	86.0	90.5

Thus the influence of the month of birth upon weight, worked out in section II, is completely supported by recorded observation of various groups of school children.

TABLE 1

The months of rapid growth related to the month of birth

MONTH OF BIRTH	ANY GIVEN YEAR PLUS THE FOLLOWING MONTHS OF AGE										
	1	2	3	4	5	6	7	8	9	10	11
January	0	0	0	0	0	0	1	2	3	4	5
February	0	0	0	0	0	1	2	3	4	5	6
March	0	0	0	0	1	2	3	4	5	6	6
April	0	0	0	1	2	3	4	5	6	6	6
May	0	0	1	2	3	4	5	6	6	6	6
June	0	1	2	3	4	5	6	6	6	6	6
Total	0	1	3	6	10	15	21	26	30	33	35
July	1	2	3	4	5	6	6	6	6	6	6
August	1	2	3	4	5	5	5	5	5	5	5
September	1	2	3	4	4	4	4	4	4	4	5
October	1	2	3	3	3	3	3	3	3	4	5
November	1	2	2	2	2	2	2	2	3	4	5
December	1	1	1	1	1	1	1	2	3	4	5
Total	6	11	15	18	20	21	21	22	24	27	31
Difference in totals	6	10	12	12	10	6	0	4	6	6	4

Example: At 12 years and 7 months of age, boys born in July will have 5 more months of rapid growth than boys born in January (6–1).

ELECTRICAL PHENOMENA OF THE BODY DURING SLEEP

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Certain electrical phenomena of the human body are frequently demonstrated by the method of Féré (1888). This method uses the body as one arm of a Wheatstone bridge through which a unidirectional current, usually from 2 to 4 volts, is passed. Measurements obtained in this way have long been termed measurements of *ohmic resistance*. Gildemeister (1915), using an electrical system which measured changes in the electrical phenomena of the body when an alternating current and a direct current were simultaneously applied, demonstrated that the so-called psychogalvanic response was not a change in *ohmic resistance*, but a change in the "polarization" of the body. That is to say, measurements made by the method of Féré are not simply measurements of resistance, but of resistance plus the polarization effect of the passage of a unidirectional current and a counter-electromotive force set up in the skin.

Waller (1918), Farmer and Chambers (1925) and also Richter (1926) report that the *apparent resistance*[2] of the body during sleep shows a very marked increase on going to sleep and a sharp fall on awakening. Richter (1926) has termed this change a very satisfactory measure of the depth of sleep. In view of these claims, it seemed worth-while to investigate electrical changes during sleep with especial regard to the following points: 1, the validity of these electrical variations as signs of other phenomena of sleep; 2, a closer analysis of the actual electrical changes taking place, and 3, the relation of the apparatus and technical procedure to the results obtained.

In all physiological galvanometry the relationship between the point of attachment of the electrodes and the results obtained is important. In general it has been shown that, when the electrodes are attached to the external skin surfaces, those portions of the skin which contain the greatest number of sweat glands give the most clearly defined electrical changes.

The sort of electrodes to be used is a question of first importance that

[1] Simmons Fellowship for the Study of Sleep.

[2] Measurements obtained by the method of Féré will be designated as "apparent resistance."

has not been satisfactorily solved. The size, method of attachment, constitution (whether liquid, paste or solid), inherent resistance, and tendency to polarization all bear a definite relation to the nature of the results one obtains; a relation which has been frequently overlooked.

The extent of skin which the electrodes cover will have its effect, also. Electrodes covering large areas will give smaller ohmic resistance determinations and show less variability to exciting stimuli than will electrodes covering only small areas. If the electrodes are not held firmly in contact with the skin, so as always to cover the same surface, slight bodily movements will cause variations in the position and area of contact, and hence galvanometric deflections occur which are not found when the electrodes are firmly fixed. Waller (1918) has demonstrated that muscular movements are not accompanied by galvanometric deflections, if other conditions are controlled.

The inherent resistance of the electrodes themselves seem to have an effect on the results. Electrodes of low resistance give the most clearly defined results. The extent to which the electrodes are subject to polarization and the constancy of the rate of polarization will, of course, affect the results.

Richter (1926), in his investigation of electrical changes during sleep, used electrodes of zinc, covered with a paste made of kaolin and saturated zinc sulfate solution. He asserts that these electrodes are non-polarizable, make an intimate contact with the skin, and are easily attached and removed. In the experimental work described in this paper we have found that many of the electrodes referred to in other contributions to the literature, including those of Richter, showed unmistakable signs of polarization. This was first evidenced by the fact that, with the reversal of the polarity of the applied current, there was a coincident fall of apparent resistance, followed by a slow building-up process covering from five to fifteen minutes.

David (1922) demonstrated that the passage of a unidirectional current through the body results in the building-up of a counter skin voltage of 80 to 98 per cent of the applied current. Hence it was thought that the fall in resistance which we found on reversal of the external polarity might be due to the discharge of this counter-electromotive force. To check this point, we tested out the various types of electrodes by the "salt-bridge" method, to find to what extent the electrodes themselves (not in contact with the body) were subject to polarization. The "salt bridge" method consists in the connecting of the electrodes by means of a U-tube filled with either physiological saline solution or the liquid which constitutes part of the electrode. The type of electrode described by Richter (1926), measured by this method, showed an initial apparent resistance of 3888 ohms, which fell at a gradual, constant rate to 3677 ohms in $9\frac{3}{4}$ hours. Measurements of the same electrodes by the alternating current method,

which obviated polarization, gave an initial resistance of 4390 ohms, which held constant for $9\frac{3}{4}$ hours. Zinc plates covered with a kaolin paste of physiological saline solution (0.9 per cent Na Cl) connected by a U-tube filled with the same saline gave a rapid, irregular increase in apparent resistance. Liquid electrodes of physiological saline in which were placed battery jars containing a saturated solution of zinc chloride and a zinc rod connected to the terminals of one arm of a Wheatstone bridge, exhibited a gradual increase in apparent resistance of 1000 ohms in 45 minutes. Measured by the alternating current method, there were no changes.

In addition to this polarization effect, it was found that the paste electrodes dried slowly when in contact with the body and that this drying caused a marked increase in the apparent resistance. If the electrodes were moistened by the addition of liquid to the paste, there was at once a pronounced fall in the apparent resistance. When two zinc plates were separated by a $\frac{1}{8}$-inch layer of kaolin-zinc sulfate paste, and the paste allowed to dry while a unidirectional current of 2 volts passed through the system, there was an initial apparent resistance of 281 ohms, which fell irregularly to 125 ohms during two hours, increased to 204 ohms in the next six hours, and then fluctuated between 116 and 271 ohms the following six hours. Plates similarly arranged but measured by the alternating current method showed no alteration in resistance for three hours, following which the resistance increased at a constant rate to eight times the original value at the end of 14 hours.

This survey of technical methods indicated quite clearly that polarization and drying of the electrodes plays an important part in diurnal variation of the apparent resistance of the body. It also brought out that the alternating current method would give determinations of true ohmic resistance free from polarization effects. A really satisfactory and accurate method of using alternating current was obtained only after considerable experimentation. An attempt was made to use the ordinary Wheatstone bridge with a fixed crystal rectifier in the galvanometer line, so that the ordinary D'Arsonval moving coil galvanometer could be used in connection with a 2-volt, 60-cycle, alternating current. This is essentially the method employed by Gildemeister (1915). For our purposes the method proved unsatisfactory because of the inductance and capacity of the resistance units used. The Kohlrausch bridge, which makes use of a telephone receiver instead of a galvanometer, was found to be unsatisfactory because of inaccuracies caused by distortions of the electrical wave and extra harmonics set up within the system. Finally, in order to make determinations of ohmic resistance free from the effects of polarization, inductance, capacity and distortion, we made use of the system shown diagrammatically in figure 1.

The A. C. source was the commercial lighting circuit of 110 volts. This

current was led to the galvanometer, a Leeds and Northrup pointer type 2370-c, with a built-in voltage drop to 3 volts.[3] The field coils of this galvanometer are in direct connection with the A. C. source and hence the readings are free from extra-harmonic effects. Two arms of the bridge were formed by a Leeds and Northrup type 4258, Kohlrausch slide wire, the galvanometer being in connection with the sliding contact. A fixed resistance unit of 20,000 ohms, designed so as to be free from inductance and capacity, formed the third arm of the bridge, while the subject formed the

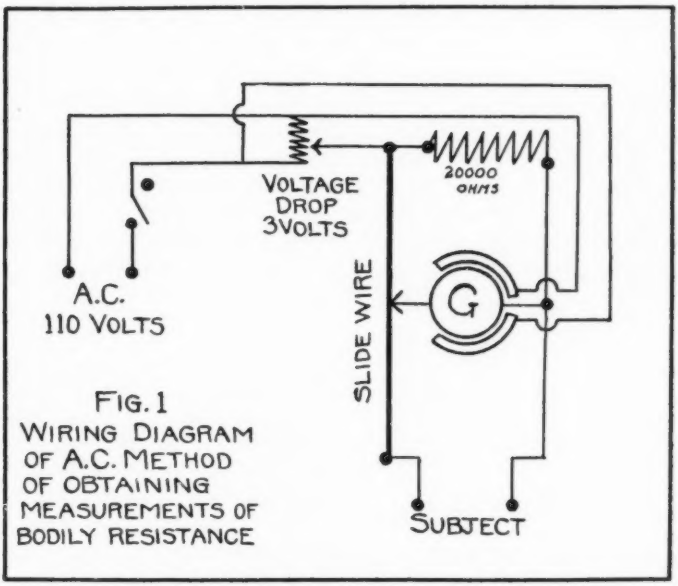


FIG. 1
WIRING DIAGRAM OF A.C. METHOD OF OBTAINING MEASUREMENTS OF BODILY RESISTANCE

fourth arm. The use of a resistance unit of 20,000 ohms was found to give discriminatory readings over a range of 2000 to 30,000 ohms, within which limits we found most bodily determinations to fall.

Electrodes were made as follows. A bit of copper wire gauze was cut to an oval shape, approximately 3 by 1 cm., the connecting wire being soldered, to one end. Two such plates were then covered with a paste made of kaolin and physiological saline. One of the electrodes so formed was placed between the second and third toes of each foot. Small surgical

[3] In all A. C. measurements the current was applied only ong enough to make the determination. It was previously found, however, that a continual flow of the current did not affect the resistance.

sponges were soaked in physiological saline and placed above and below each electrode. The toes, sponges and electrodes were then wrapped with a short bandage of oiled silk, and this in turn was enveloped with cotton gauze. It was found that electrodes so arranged did not dry sufficiently within eight hours to affect the resistance. Further, these electrodes were found to be comparatively comfortable and free from annoying effects.

A copper-constantine thermo-couple was placed in the paste of one electrode and connected, together with the cold junction, to a high sensitivity galvanometer adjusted so that very slight variations in skin temperature (0.01°C.) were demonstrable.

The bed in which the subject spent the periods of sleep was suspended on four gimbal supports, so that it swung freely. An electrically driven kymograph was placed by the bed and a pen attached to the bed was so arranged as to record on a moving paper tape all major movements of the person resting on the bed. Slow movements of the whole arm, or sudden movements of the forearm were near the limit of sensibility of the system.

EXPERIMENTS WITH THE ALTERNATING CURRENT. The use of the A. C. method of measuring the diurnal variations in bodily resistance demonstrated several very interesting facts. The findings of the experiments conducted during the day while the subject was awake are summarizable follows.

Experiment D 1. Subject C. L. August 8, 1926. Electrodes were composed of copper gauze covered with a kaolin-physiological saline paste; no sponges to prevent drying. Electrodes placed between second and third toes of each foot. External current, 3 volts A. C. Initial resistance, 5300 ohms. Resistance increased in an irregular fashion to 8565 ohms in thirty minutes. During the next 47 minutes resistance averaged 8470 ohms with a range between 7825 ohms and 8900 ohms. Subsequent to this the resistance showed a constant increase due to the drying of the electrodes.

Experiment D 2. Subject C. L. August 9, 1926. Electrodes same as in D 1, except sponges soaked in physiological saline were added so that there was not enough drying during the course of the experiment to affect the resistance. Applied current, 3 volts A. C. Initial resistance, 4700 ohms, increasing irregularly to 5000 ohms in 40 minutes. During the following 392 minutes the resistance averaged 5135 ohms, ranging between 5000 and 5640 ohms.

Experiment D 3. Subject C. L. August 13, 1926. Electrodes and applied current same as in D 2. Initial resistance, 6640 ohms; final resistance, 270 minutes later, 7900 ohms, irregular course. Average resistance, 6765 ohms; range, 5955 to 7900 ohms.

Experiment D 4. Subject C. L. August 22, 1926. Electrodes and applied current same as D 2. Initial resistance, 4390 ohms; final resistance, 5640 ohms after 190 minutes. Average resistance, 5087 ohms; range from 4390 to 5640 ohms. Temperature of one electrode was taken during the same period and showed an irregular course bearing no relation in direction of change to that of changes in resistance. Coincident readings of apparent resistance using a 2 volt D. C. current applied only at the periods of determinations (10-minute intervals). The apparent resistance

started at 5430 ohms, and 190 minutes later was 10,600 ohms. The increase in resistance was constant in rate.

Experiment D 5. Subject C. L. September 5, 1926. Applied A. C. 3 volts. Electrodes, copper gauze covered with kaolin-physiological saline paste. The electrodes were placed between the second and third toes of each foot every 15 minutes and a resistance determination made. Subsequent to the first determination the feet were placed in vessels containing physiological saline and were removed only long enough to make later determinations. The initial reading was 10,500 ohms, followed by 7700, 6232, 5640, 5480, 5480, 5480 and 5480 ohms, 15 minutes elapsing between each determination. After the last reading, while the electrodes were still in place a needle held in an insulated holder was run through the paste and the skin into the flesh of the toe. Resistance immediately dropped to 4390 ohms, no change being noted when the needle was removed. Piercing the toe of the opposite foot caused a fall to 4200 ohms. During two next minutes the resistance rose to 4850 ohms. Tincture of iodine (83 per cent alcohol) was then poured over the electrodes and toes. Within six minutes the resistance fell to 4800 ohms and in 7 minutes following to 3810 ohms, which point remained constant for subsequent thirty minutes. The iodine irritated the skin so as to form blisters.

The course of the resistance changes during the period of sleep is shown graphically in figure 2. The dots on the curves represent readings taken just after movements were made by the sleeper. The experiments were as follows, the numbers of the experiments being the same as the numbers of the curves in figure 2

Experiment 9. Subject A. L. August 10, 1926. Applied current and electrodes same as in D 2. Course of resistance changes shown in curve *9* of figure 2.

Experiment 10. Subject A. L. August 11, 1926. Applied current and electrodes same as in D 2. Course of resistance changes shown by curve *10* of figure 2.

Experiment 11. Subject A. L. August 22, 1926. Applied current and electrodes same as in D 2. Course of resistance changes shown by curve *11* of figure 2. Course of temperature changes of the electrodes shown by curve *11* of figure 3.

Experiment 12. Subject G. E. W. August 23, 1926. Applied current and electrodes same as in D 2. Course of resistance changes shown by curve *12* of figure 2.

Experiment 13. Subject G. E. W. August 24, 1926. Applied current and electrodes same as D 2. Course of resistance changes shown by curve *13* of figure 2. Course of temperature changes shown by curve *13* of figure 3.

These experiments indicate the following facts.

1. The ohmic resistance of the body during the period of sleep holds a constant or a very nearly constant level after a period of adaptation of some kind, to the electrodes.

2. The period of adaptation is very probably what Waller (1903, p. 122) demonstrated and described as "simply capillary currents arising at the surface of separation between the salt solution and the skin." It is not due to a changing relation between the temperatures of the skin and of the electrodes.[4]

[4] In an article by Lewis and Zotterman, 1927, Jour. of Physiol., lxvii, 280, which has appeared since this report was sent to press it has been shown that the adaptation change is due to changes in the outside horny layer of the epidermis.

3. During the day the body shows a certain amount of variation in resistance. This variation is not nearly so great as the previous studies (Waller, 1918), made with a unidirectional current, indicate.

4. Several investigators have suggested that practically the entire resistance of the body lies in the epidermal layers of the skin. Our experiment of running a needle through the electrode and skin, so that contact was made directly with the subcutaneous tissues, with a consequent drop of only one-fifth of the total *resistance*, indicates that the major portion of

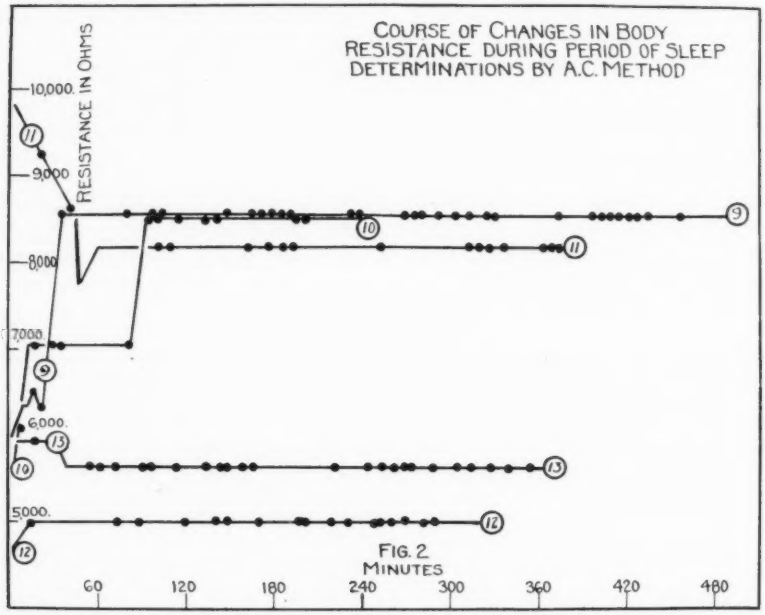


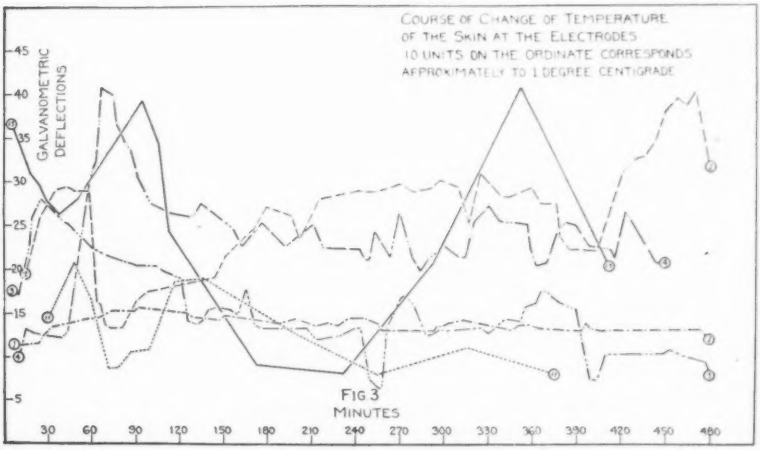
FIG. 2

the resistance of the body is not at the skin. As compared to Richter's (1926) similar experiment, it indicates that the greater extent of *polarization* does occur at the skin surfaces.

5. Crile (1926, p. 103) says: "Iodine increases the electrical conductivity of living tissue; iodine increases permeability." Ebbecke (1921) found that chemical irritation of the skin produced an increased apparent resistance strictly limited to the area stimulated. Our results in experiment D 5 confirm Crile's statement and controvert Ebbecke's. Possibly the increase in the conductivity function outweighs the result of the irritation.

6. There is no close correspondence between skin temperature and resistance as will be seen by a comparison of the curves of figures 2 and 3. Waller (1918) reports a close correspondence between "clinical temperature" of the body and apparent resistance. Our comparison is between actual ohmic resistance (in this case) and skin temperature at the electrode, while his is between resistance plus depolarization and counter-electromotive force on the one hand, and "clinical temperature" on the other. We interpret the expression "clinical temperature" as meaning the sublingual temperature.

EXPERIMENTS WITH THE UNIDIRECTIONAL CURRENT. Waller (1918), in an attempt to evaluate the significance of the psychogalvanic reflex under



varying conditions, took hourly or bi-hourly readings of his own bodily resistance over several periods of five consecutive days. His results indicated that the apparent resistance of the body fluctuates rather widely, showing a marked increase during the early portion of the night and a decline during the early morning hours to a minimum about 8 a.m. Since he personally made the measurements on his own body, the increase in resistance which he found was not attributable to sleep, as some other writers, referring to his work, have supposed. Slight (1926) using technique which was an improvement on Waller's, obtained curves of diurnal variation in polarization which are of the same form as the curves described by Waller. Peiper (1925), working with infants, found that the so-called psychogalvanic reflex could not be elicited during deep sleep. He also states that with each stage of the depth of sleep there are irregular

deviations (undulatory) in sweat gland innervation which are to be explained as plethysmographic waves of the third order. He estimated depth of sleep by the intensity of acoustic stimulation necessary to awaken the sleeper. Farmer and Chambers (1925), working with three subjects, found:

"There was a very great rise in resistance during sleep. The rise was very rapid at the beginning of the night and rose to a peak immediately before getting up in the morning. The drop after being awakened was as rapid as the rise of the preceding night. . . . The subject who had the highest degree of emotionality, estimated both subjectively and objectively, had the deepest sleep with no galvanic responses of any kind, his resistance during sleep being a perfectly straight line at a resistance of 32,000 ohms. The least emotional of the three subjects had the largest number of galvanic responses during sleep, and had a varying resistance, reaching its highest point at 9,400 ohms (*sic*) . . . . One of the subjects was aroused during the night and so had his sleep broken, his resistance at once fell to the normal level of his waking period; and also another subject fell asleep in the daytime and his resistance immediately rose; so that we can conclude that the high resistance during the night was definitely due to sleep and not to diurnal rhythm (*sic*)."

In the opinion of the writer, the data, together with such description of technical methods as are given by Farmer and Chambers, do not indicate, as they argue, that sleep is the cause of the rise in resistance. One of the three subjects, as they themselves say, showed a constant resistance during the night, while two subjects showed an increased *apparent* resistance. Certainly they must offer more evidence and a clearer description of their technique in order to support their conclusion. Richter (1926), summarizing his experimental work measuring the changes in apparent resistance during sleep of 16 subjects, says: "We have found in all cases without exception the palm-palm record increased, usually quite markedly," during sleep. He presents only one graph or report of actual figures. This shows an initial resistance of 70,000 ohms at 9 p.m., mounting to 1,400,000 ohms at 3 a.m., and falling to 120,000 ohms on awakening at 8 a.m. The graph indicates that his readings were made at intervals of about one hour. He says:

"Because the individual variations are too great we are not able to give an average normal curve for the intensity of sleep. . . . . This close correspondence between the depth of sleep and the resistance is shown further very clearly by the records of the following experiments. It was found that awakening, however suddenly brought about, is followed instantly by a drop of the resistance from any level to the waking level. . . . . It was found that the changes in resistance of the palms follow closely the depth or intensity of sleep. As soon as the individual drops off to sleep the resistance begins to increase." (So far as one can tell from his report, his measure or standard of depth of sleep, other than the electrical change, must have been simply looking at the sleeper.) "This, then, offers a new method of measuring the depth of sleep and has the marked advantage that its successful use does not require the interruption of sleep."

Our experiments were conducted on one subject on the nights of August 7, 12, 13, 14, 15 and 20, 1926. The applied galvanic potential was 2 volts which was allowed to flow continuously. Determinations were made by the use of an ordinary Wheatstone bridge and a high sensitivity D'Arsonval galvanometer. Our electrodes were made of zinc plates, 2 inches in diameter, covered with a paste made of kaolin and a saturated solution of zinc sulfate. In place of attaching our electrodes to the palms, as Richter did, we attached them to the soles of the feet, since this method of attachment interfered less with the movements of the sleeping subject. Readings were ordinarily taken at intervals of 5 minutes early in the night and 15 minutes

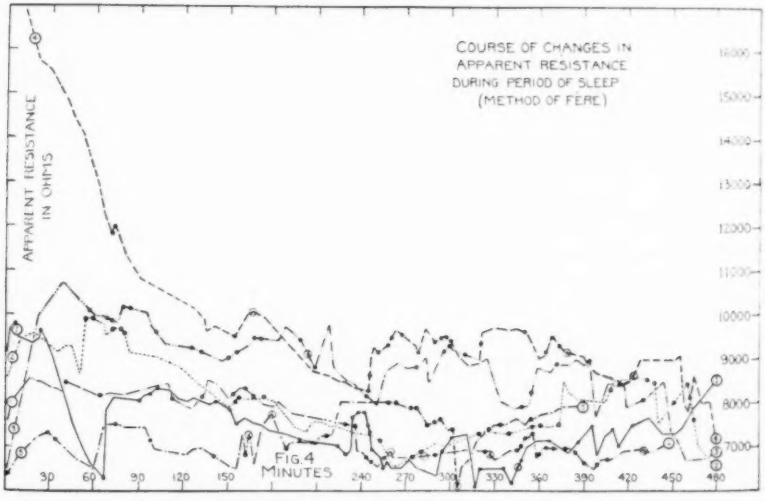


FIG. 4

during the latter part of the night, but additional readings were interpolated whenever the subject stirred in bed. The subject was instructed to say "awake" at any time during the night on awakening.

The results of our experiments are shown graphically in figure 4. The dots on the curves indicate readings taken just after movements made by the sleeper. The circles containing crosses represent readings taken immediately after the subject announced "awake." Curves *1*, *2*, *3* and *4* of figure 3 represent the course of change in skin temperature coincident with the apparent resistance curves *1*, *2*, *3*, and *4* of figure 4.

The following observations were made during the course of these experiments:

1. Going to sleep, *in the case of this subject*, was not always accompanied by a rise in apparent resistance (see, *e.g.*, curve *4*, fig. 4).

2. Awakening was not always accompanied by a drop in apparent resistance (see *e.g.*, fig. 4, curve *1*; curve *3* at the 452nd minute; curve *5* at the 180th minute).

3. There is no systematic correspondence between the degree of postural activity and the amount or the direction of change of the galvanometer reading. The changes of position of the subject are not systematically accompanied by deflections of the galvanometer.

4. These variations in apparent resistance are not variations in actual ohmic resistance, as experiments 9, 10, 11, 12 and 13 have shown. They must be variations due either to polarization, a building-up or discharging of a counter-electromotive force at an irregular rate, or to instrumental factors, such as to polarization of the electrodes themselves, drying of the electrodes, irritation of the skin by the surface of the electrode, etc.

5. Bujas (1922) stated, on *a priori* grounds, that the psychogalvanic reflex as well as other variations in apparent bodily resistance were due to temperature changes between the skin and the electrodes. We found a direct contradiction to this assumption; namely, no relation between changes in apparent resistance and the temperature of the skin at the electrodes. A few casual experiments in which the psychogalvanic reflex was obtained, while coincident temperature records were taken, failed to show any correspondence in the two sets of readings.

Conclusions. The purpose of the original experiments of Kohlschütter (1862) and of subsequent experiments by various workers was to measure the depth of sleep in terms of the intensity of the stimulus necessary to awaken the subject. As Karger (1925), Richter (1926) and others have pointed out, such a method suffers the disadvantage of tending to modify the course of sleep itself, so that the variable under measurement is distorted by the means employed to measure it. An additional fallacy, which is common not only to these experiments on sleep but also to a large number of other physiological and psychological experiments, is the fallacy of taking one single variable and calling it an index of the total integration of a large number of factors whose combined action has not been subjected to simultaneous measurement and compared with the "index." The designation of the index as such is arbitrary and confusing. Such terms as "depth of sleep," "emotionality," "fatigue," etc., are descriptive of the sum total of numerous inter-related factors which balance and vary among themselves.

Consider the implications of the phrase "depth of sleep." As used in a subjective description, it implies that the period of sleep was restful, that the sleeper was not disturbed, and that he did not dream or is unable to remember his dreams. Objectively, we customarily connect the phrase with a slow, deep, regular respiration, with an absence of postural activity,

with the intensity of stimuli necessary to awake the sleeper, with an absence of somnambulism and verbalization, with a marked lowering of tonicity of skeletal musculature, with a drop in body temperature, with an added electrical conductivity of brain tissue (Crile, 1926), with a lowered metabolic rate, the abolition of certain reflexes, with a dominance of parasympathetic activity (Rosenbach, 1880), and so on.

It is true that almost any one of the factors mentioned above may be increased or decreased quite markedly without necessarily altering the sleep process. One might sleep quite soundly while the metabolic rate was very high, or with several degrees of fever. The use of several of the barbituric acid derivatives as hypnotics (barbital, dial, etc.) is frequently accompanied by a marked increase in postural activity, though the sleep is reported later by the subject as having been very sound. Possibly it may be true that sleep, strictly speaking, is a cerebral phenomenon. Crile's theory (1926) that sleep is a repolarization of the central nervous system is suggestive but lacks experimental verification.

If on *a priori* grounds one grants that there is an integration of bodily functions which might be called "depth of sleep," then we must concede that at present we know of no adequate single criterion or team of criteria which can measure such an integration. On strictly logical grounds it is probably unwarranted to use such general terms as "depth of sleep," "intelligence," etc., in a scientific discussion. Johnson, Swan and Weigand (1926) have given an extended treatment of this point in a recent review of the literature on the topic of *Sleep*.

Acknowledgments. This experimental study was subsidized by a grant from the Simmons Fellowship for the Study of Sleep at Mellon Institute. It was undertaken at the suggestion of Dr. H. M. Johnson. During the past year Doctor Johnson and Mr. G. E. Weigand have been obtaining records of changes in position during the night of a group of experimental sleepers, and are relating their measurements to other variables. It was obvious that such records did not cover the entire scale of bodily activity during the time devoted to sleep. The reports of several experimenters suggested that the variations in electrical phenomena of the body might be used as indicators of changes not covered by the tests already in use. Hence it was desired that these phenomena should be investigated as a possible addition to the present program of experimentation.

The writer wishes to record his indebtedness to Dr. H. M. Johnson, Dr. Thomas Swan and Mr. G. E. Weigand for valuable aid and suggestions. He also acknowledges his appreciation of the aid of Prof. O. Blackwood, of the University of Pittsburgh, and of Mr. H. N. Stroh, of the Electrical Instrument Service Company, Pittsburgh, in devising the electrical systems used.

SUMMARY

Several experiments were conducted bearing on the following general points: 1. The validity of variations in bodily electrical phenomena as measures or criteria of the "depth of sleep." 2. An attempt at a closer analysis of the electrical responses of the body with respect to their actual electrical nature, resistance, polarization, potential, etc. 3. The relation of the apparatus and technical procedure to the results obtained.

Our results may be summarized as follows.

1. All the "non-polarizable" electrodes which we tested were found to polarize quite appreciably when tested by the salt bridge method using a unidirectional current of 2 volts.

2. Drying of paste electrodes causes a marked increase in the resistance of the electrodes themselves.

3. An electrical system using a 60-cycle alternating current was devised which gave determinations of true ohmic resistance free from the effects of polarization, inductance and capacity, and from drying of the electrodes over a period of eight hours.

4. After a period of physical accommodation to the electrodes there is little or no change in the true resistance of the body during sleep.

5. One experiment indicated that only about one-fifth of the total bodily resistance lies in the skin. Iodine applied to the skin resulted in a decreased resistance.

6. During the day the body shows a certain amount of variation in actual resistance which is less than previous studies, making use of a unidirectional current, have indicated.

7. There is no correspondence between the course of skin temperature at the electrodes and the electrical changes of the body.

8. Experiments using a unidirectional current and measuring the apparent bodily resistance failed to confirm the statements of Farmer and Chambers and of Richter that such changes were measures of the quality or depth of sleep.

9. There is no correspondence between the frequency or amount of postural activity during the sleep period and the electrical changes of the body.

10. A criticism of the use of single physiological or psychological factors as adequate measures or criteria of complicated reactions is offered.

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THE EXPERIMENTAL FEEDING OF FRESH ANTERIOR PITUITARY SUBSTANCE TO THE HYPOPHYSECTOMIZED RAT[1]

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Aside from papers by Dott (1923 a, b) there appear to be no experiments reported upon the feeding of pituitary substance to the hypophysectomized mammal. Dott found that the subnormal temperature and lethargy of his dogs were relieved by pituitary feeding but that their failure to grow was not influenced nor was the histological appearance of their degenerated epiphyseal cartilages altered. However, since his completely hypophysectomized dogs did not survive for more than fourteen days it is possible that the treatment was too brief for beneficial effects upon the growth retardation and upon the genital atrophy to become evident. The brief survival of his hypophysectomized dogs with the consequent limitation in the time of treatment, is unfortunate for it would seem that a pituitaryless animal which survived over an extended period would have certain distinct advantages over a normal for testing the efficacy of pituitary feeding.[2]

Hypophysectomized rats are valuable for this test. They can be secured in adequate numbers for I have shown (Smith, 1926, 1927) that the pituitary is readily ablated through a ventral approach.[3] Rats hypophysectomized in this manner exhibit a complete, or nearly complete growth stasis and a profound atrophy of the genital system, adrenal cortex and thyroid. They live for months. It is obvious that because of the growth stasis displayed by these animals any effect upon growth would be more readily discernible in them than it would be in the normal where the growth rate is rapid and shows considerable variability.

[1] Aided by a Grant from the Research Board of the University of California, where most of the work upon this problem was done.

[2] Many experiments with pituitary feeding in the normal animal have been reported. In the majority of cases this feeding has had either no effect, or possibly a retarding influence upon growth and development (Aldrich 1912a, b; Schafer, 1912; Lewis and Miller, 1913; Frank, 1919; Sisson and Broyles, 1921; Evans and Long, 1921; Drummond and Caanan, 1922; C. S. Smith, 1923). In a few cases a stimulation of growth and development is reported (Schafer, 1911; Robertson, 1916 and earlier; Goetsch, 1916; Marinus, 1919; Dott, 1923).

[3] The anterior and the posterior (neural and intermediate) lobes only are ablated. The pars tuberalis and the pituitary stalk remain intact.

The hypophysectomized rat would also appear to be preferable to the normal because it responds more readily to certain kinds of pituitary administration. This is shown by the immediate stimulation of growth and by the response of the thyroid, adrenal cortex and genital system which is induced by daily pituitary homeotransplants as compared with the very slight, or failure of, response, save in the genital system, of the normal to this treatment. While this increased response of the hypophysectomized rat as compared to the normal may be due to the fact that the presence of a physiological deficiency aids in a "take" of the transplants, results gained with the thyroid indicate that this is not the sole reason. The retardation in the rate of growth of the thyroidectomized rat can be remedied by the intraperitoneal injection of an amount of thyroid extract which is too small to alter the growth of the normal rat. Their basal metabolic rate, also, as shown by some unpublished work of G. L. Foster is increased more by this dosage than is that of the normal. Thus from the fact that the pituitaryless rat is much more responsive to the daily pituitary homeotransplants than the normal, and from analogy with the results gained in thyroid treatment, it would seem that the hypophysectomized rat should be more responsive to the oral administration of pituitary than the normal. A series of experiments was consequently begun to determine whether the daily feeding of fresh pituitary substance would have any beneficial effects upon the disabilities displayed by the hypophysectomized rat.

Experimental procedure. Rats (pied) which had been hypophysectomized for varying periods of time (see table and figure) were each fed daily with two bovine anterior pituitaries. The glands were fed a short time before the standard diet was placed in the cages. It was made certain that the glands were eaten. The pituitary glands were secured daily fron the abattoir, and were usually fed not longer than six hours after the beef had been slaughtered. Two types of controls were used: 1, normal unoperated littermates supplied with the standard diet; 2, hypophysectomized littermates which in addition to the standard died were fed fresh muscle in amounts equal to two anterior pituitaries. The animals were weighed every three days. Body and tail lengths were measured every two weeks, each animal being placed under deep ether narcosis to insure complete relaxation.

Experimental findings. The growth stasis displayed by pituitaryless animals was not modified by the anterior pituitary feeding extending over a period of some months, they remaining stationary in weight save for the usual slight fluctuations. Their growth curves thus parallel those of the hypophysectomized controls receiving muscle as a control substance. Their body and tail lengths showed only a slight increase characteristic of animals hypophysectomized during their growing period, and consequently

The results obtained from feeding fresh anterior pituitary of the ox to hypophysectomized rats

DESIGNATION OF RAT	TYPE	SPECIAL SUBSTANCE FED	EXPERIMENT BEGUN*			SPECIAL FEEDING BEGUN†			EXPERIMENT TERMINATED						WEIGHT OF BOTH		
			Age	Weight	Length	Age	Weight	Length	Age	Weight	Length	Number days special feeding	Change during special feeding in Weight	Change during special feeding in Total length	Gonads	Adrenals	Thyroids
GH 687 ♀	Control	None	48	114	290	59	136	312	165	223	364		87	52			
BH 688 ♀	Hypophysectomized	Anterior hypophysis	48	122	295	59	106	299	165	128	303	106	22	4			
W 739 ♀	Control	None	73	118	313	77	136	321	146	189	356		53	35	0.0665	0.0515	0.0335
W 735 ♀	Hypophysectomized	Anterior hypophysis	73	129	314	77	126	315	146	121	319	69	−5	4	0.0170	0.0090	0.0100
BH 1239 ♀	Control	None	53	140	312	85	175	345	189	213	371		38	26			
W 1241 ♀	Hypophysectomized	Anterior hypophysis	53	156	326	85	120	328	189	115	328	104	−5	0	0.0096	0.0055	0.0098
G 1243 ♀	Hypophysectomized	Muscle	53	142	315	85	128	316	189	120	316	104	−8	0	0.0112	0.0098	0.0122
W 1274 ♀	Control	None	45	107	284	56	144	310	147	230	386						
W 1275 ♀	Hypophysectomized	Anterior hypophysis	45	119	297	56	109	300	147	107	301	91			Still living		
W 1276 ♀	Hypophysectomized	Muscle	45	115	294	56	115	298	147	120	300	91			Still living		
W 1289 ♂	Control	None	38	101	275	46	140	291	143	322	419		182	128	3.4500	0.0480	0.0390
W 1287 ♂	Hypophysectomized	Anterior hypophysis	38	109	278	46	100	282	143	95	286	97	−5	4	0.1390	0.0056	0.0060
BH 1291 ♂	Hypophysectomized	Anterior hypophysis	38	119	287	46	102	290	143	104	292	97	2	2	0.1588	0.0052	0.0057
W 1288 ♂	Hypophysectomized	Muscle	38	112	277	46	102	279	101	92	283	55	−10	4	0.1502	0.0060	0.0070

* The hypophysectomized animals were operated upon at the age, weight, and total length given in this column. The weight and length of the unoperated controls is given for comparison.

† For comparison the weight and length of the unoperated controls is also given in this column although they received no special diet.

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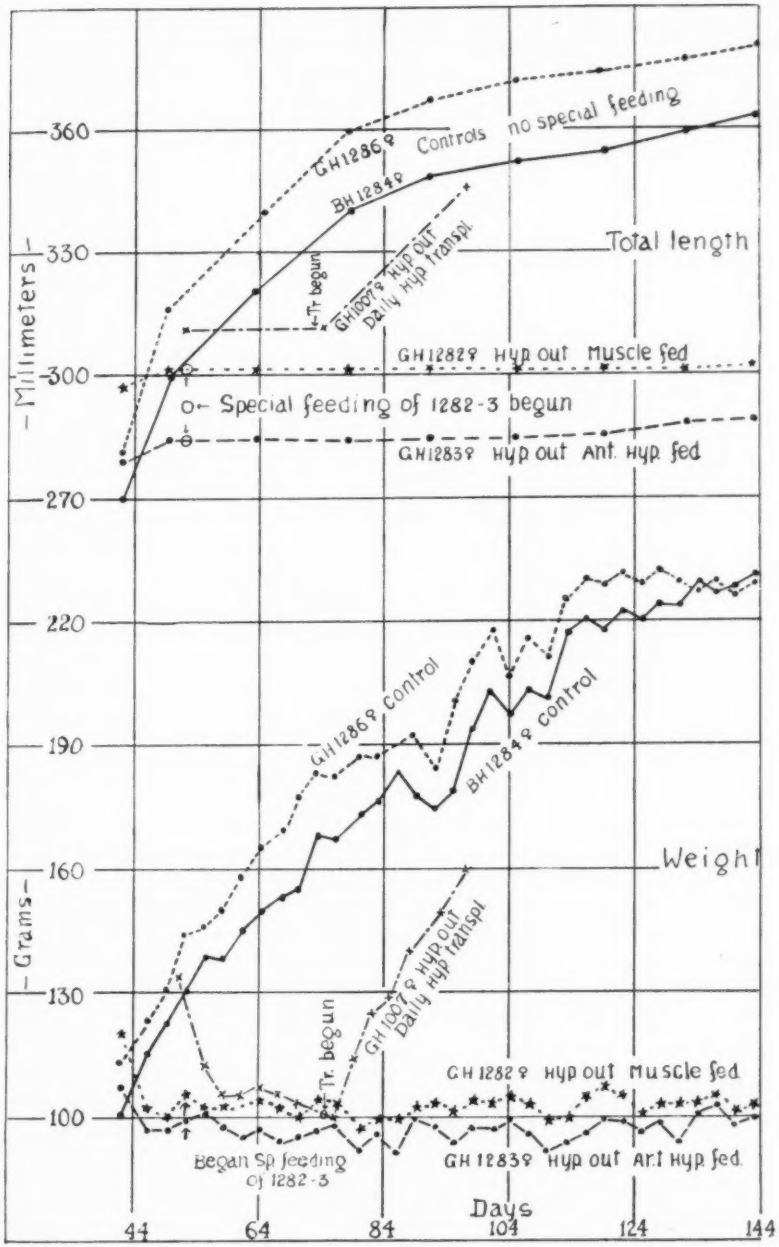

Fig. 1

curves showing their total lengths parallel those of their pituitaryless mates receiving muscle as a control substance.

Autopsy of some of the animals at the termination of the experiment revealed no response of the genital system, the thyroids or the adrenal cortex. These displayed the atrophy typical of the hypophysectomized rat. In this experiment I have not fed the posterior pituitary in addition to the anterior. That this would not have been beneficial is shown by at least two lines of evidence: 1, the ablation of the posterior pituitary does not alter the normal growth rate nor impair the sex apparatus or endocrine glands of the rat, results which are in harmony with those reported by other investigators upon other animals. 2, I have reported that the injection of a fresh bovine anterior pituitary suspension (that of Evans and Long, 1921) induces a growth response in hypophysectomized rats, but fails to repair their atrophied sex and endocrine systems. The addition of fresh posterior pituitary does not make the injections more effective.

In order to contrast the absence of a response to pituitary feeding with the profound response elicited by daily pituitary transplants, I have added in the accompanying figure the growth curve of a hypophysectomized rat (not a littermate) receiving the transplants.

Discussion. The results of these experiments seem to show conclusively that the oral administration of fresh bovine anterior pituitary exerts no beneficial effects upon any of the disabilities induced by hypophysectomy in the rat. This is not due to any incapacity of the animals to respond for as I have shown elsewhere they respond promptly to daily pituitary homeotransplants. Indeed because of their physiological deficit they should respond more readily to a replacement therapy than should the normal animal with its balanced endocrine system.

Several reasons might be advanced to explain this failure of pituitary feeding to elicit a response. It seems advisable to examine these briefly. One is that the amount of pituitary fed was insufficient. Yet the amount fed exceeds that employed in most of the experiments with the feeding of endocrine glands, and greatly exceeds the amounts administered in clinical cases, in many of which beneficial effects have nevertheless been claimed. With the feeding of a very much larger amount of pituitary tissue its nutritive value would possibly become of sufficient importance to affect the results.

Although the pituitary substance fed was relatively fresh, it may be claimed that autolytic or other changes may have destroyed its hormone, and that the method of administration was not responsible for the failure to secure a response. But the intraperitoneal injection of emulsions made from glands such as I have fed induces an acceleration in growth in normals (Evans and Long, 1921). These injections also cause a resumption of the normal rate of growth in rats which have been dwarfed by hypophysectomy

(Smith, 1926). Moreover, a group of us have shown that this emulsion, to which a small amount of alcohol has been added, elicits a growth response when injected after days of storage at room temperature (Flower, *et al*, 1923). It is thus evident that this pituitary tissue contained a principle which can maintain and accelerate growth, yet no growth resulted when it was administered orally.

This failure also may be due to a functional difference between the anterior pituitaries of the ox and the rat. Upon this point no data are available. Comparative physiological studies upon the other glands of internal secretion in mammals, however, have revealed no important functional differences, and so it would seem unlikely that the function of this gland in these two forms is dissimilar. Yet such a possibility cannot be entirely disregarded.

Since the intraperitoneal injection of an extract or suspension made from beef pituitaries has no beneficial effects upon the atrophied genital system, thyroid or adrenals of the hypophysectomized rat, a beneficial effect upon these atrophied organs from the feeding of these glands could not be expected. However, there is a substance contained in these bovine glands which affects the gonads. Injection of an extract made from them injures the ova of the rat (Evans, 1924) and causes a diminution in the size of the testes (Evans and Simpson, 1926); and when given concurrently with pituitary homeotransplants it prevents the usual restorative effects given by these transplants in the hypophysectomized rat (Smith, 1927). That this substance which adversely affects the gonads is not subject to alimentary absorption is shown by the many pituitary experiments in normal animals. For in none of these reported do we find injurious effects upon the gonads of the mammal mentioned.

In an earlier paper (Smith, 1918) it was stated that the feeding of fresh pituitary induced a normal rate of growth in the slowly growing hypophysectomized tadpole. This form is widely separate from the rat, phylogenetically, and by the temperature at which digestion is carried on, as well as by possible differences in the digestive enzymes. This feeding experiment upon the tadpole also differed in another and perhaps more important respect from that reported here. With the experiment on the tadpole the pituitary substance constituted the entire food supply, except for a small amount of lettuce, and any difference between the nutritive value of pituitary and the control diet would be important. In the present experiment pituitary formed only a small part of the food supply and its nutritive value would not be of great importance. It should be further pointed out that in contrast to the rat the tadpole continues to grow although at a slowed rate, after hypophysectomy. Anterior pituitary feeding to the tadpole did not restore in the slightest degree the atrophied thyroid nor adrenal cortex, results identical with those obtained in the hypophysectomized rat.

SUMMARY

The daily feeding of two fresh bovine anterior pituitary glands does not cause any increase in the body weight, or in the tail or body length of the hypophysectomized rat. The growth curves of animals thus treated parallel those of the operated rat receiving an equal amount of muscle as a control substance. This anterior pituitary feeding also fails to restore, in the slightest degree, the atrophied genital system, thyroids or adrenal cortex of the hypophysectomized rat.

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THE EFFECT OF PITUITRIN ADMINISTRATION UPON CERTAIN PHASES OF CARBOHYDRATE METABOLISM

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It has been known for some time that the administration of extracts prepared from the posterior lobe of the pituitary gland may result in an apparent diminution of carbohydrate tolerance (Goetsch, Cushing and Jacobson, 1911). Burn (1923) has shown that pituitrin is apparently antagonistic to both insulin hypoglycemia and adrenalin hyperglycemia. Some degree of hyperglycemia usually follows the intravenous injection of this extract in normal animals, while at times no effect or even a hypoglycemia has been observed. Although numerous investigators have from time to time confirmed these observations, little information is available as to the mechanism involved in the action of extracts of the pituitary gland.

It was thought that additional information as to the effect of pituitary extracts on carbohydrate metabolism might be obtained from experiments designed so as to permit a more complete study of the various responses and a more carefully controlled rate of glucose and pituitrin[1] administration. With this purpose in mind we have compared the response of normal dogs to a continuous intravenous injection of glucose with and without pituitrin. The particular phases of the response studied were the changes in blood sugar, hemoglobin, plasma pH and CO_2 content, urinary volume and sugar content, respiratory quotient, heat production and body temperature occurring during and following the injection period. Control observations were also made as to the effect upon blood sugar of a continuous injection of Ringer's solution with and without the addition of pituitrin.

METHODS. The methods of study employed were essentially the same as those reported elsewhere (Boyd, Hines and Leese, 1925). Female dogs were placed on a standard diet and accustomed to the routine of the experiment. Blood was drawn from the jugular vein before and at the end of the injection period. The marginal ear vein was used as the source

[1] In this paper "pituitrin" is used to designate a commercial extract of the posterior lobe of the pituitary gland.

of blood for determinations made during and after the injection. Blood sugar determinations were made by a micro Folin and Wu method. Hemoglobin was determined according to Palmer's method. For comparison the preliminary values were designated as 100 per cent. Plasma CO_2 content was determined according to the method of Van Slyke and Cullen and pH by the method of Myers, Schmitz and Booher. Urine was collected by continuous catheterization and its sugar content determined by the method of Folin and Berglund. Air for determination of respiratory quotient and heat production was collected in a Tissot spirometer and analyzed according to Haldane. The glucose (Merck) was dissolved in distilled water and injected into the saphenous vein according to the method of Woodyatt. A 16 per cent solution was used for experiments in which the rate of administration was 1.8 grams per kilo body weight per hour and approximately a 30 per cent solution for the higher rates. The concentration of the glucose was determined by analysis previous to the injection. Commercial pituitary extracts, either the "Pituitrin" of Parke, Davis & Company, or the "Pituitary Extract, Lilly" of Eli Lilly & Company, were used in this work. The contents of the ampoules were diluted approximately 1 to 10 with normal saline and administered by the continuous intravenous infusion method described by Burn and Dale (1924). In order to avoid making a double intravenous puncture, the needle from the burette containing dilute pituitrin was inserted through the rubber tubing into the hilt of the needle through which the glucose was being administered. This method lessened the possible chances of pituitrin destruction by heat and prolonged contact with glucose solutions. The duration of the injection period was usually 2 to 3 hours and observations were made in the post-injection period for 3 to 5 hours. Except in one experiment (701 E) no anesthetic was employed.

Results. The injection of glucose and pituitrin caused a higher level of blood sugar to be maintained than in control experiments on the same animal with glucose alone. This effect was apparent early in the injection period. The form of the blood sugar curve during the injection period was not essentially modified by pituitrin administration. The return to preliminary values in the post-injection period was somewhat slower than in the control experiments. The continuous intravenous administration of Ringer's solution at the rate of 15 to 25 cc. per kilo body weight per hour was without influence on the blood sugar level. However, when from 0.05 to 0.075 cc. of pituitrin was added to this amount of Ringer's solution a small but definite hyperglycemia appeared in every case.

Table 1 summarizes the hourly and total retention of injected glucose in the various experiments. The term sugar retention was used to represent that amount of sugar injected which does not appear in the urine. It will be seen that animals which received glucose at the rate of 4 grams

per kilo per hour eliminated, on the average, 17.6 per cent of this amount in the urine. In experiments on the same animals in which pituitrin was administered simultaneously with the glucose, the amount lost in the urine averaged 36.7 per cent. In these experiments pituitrin was without

TABLE 1

Percentage of injected glucose retained—based upon total retention as compared with total amount injected up to the designated time

EXPERIMENT NUMBER	END OF FIRST HOUR INJECTION	END OF SECOND HOUR INJECTION	END OF THIRD HOUR INJECTION	TOTAL (WITH RECOVERY)	PERCENTAGE OF URINE GLUCOSE
Rate of glucose administration—4 grams per kilo per hour					
700 A Control	90.0	83.6	77.7	76.6	4.46
700 E Control	85.5	83.2	81.3	80.6	4.55
Average of controls	87.8	83.4	79.5	78.6	4.51
700 F Pituitrin	74.6	62.4	57.4	53.5	4.85
701 A Control	79.8	77.8		77.6	5.53
701 E Pituitrin				69.2	4.46
702 A Control	92.2	88.3		88.4	4.67
702 E Control	88.9	85.5		85.3	4.88
Average of controls	90.6	86.9		86.8	4.78
702 G Pituitrin	63.2	60.4		58.9	3.80
703 A Control	84.6	81.3		80.9	4.51
703 D Control	92.0	92.4		92.5	2.92
Average of controls	88.3	86.9		86.7	3.72
703 I Pituitrin	79.1	72.5		72.4	5.55
Average Control	86.6	83.8		82.4	4.64
Average Pituitrin	72.3	65.1		63.5	4.67
Rate of glucose administration—1.8 grams per kilo per hour					
710 A Control	94.5	91.6		90.9	3.84
710 B Pituitrin	89.9	83.5		82.8	5.19
711 A Control	87.3	87.9		87.7	6.08
711 B Pituitrin	76.6	65.2		62.0	7.65

influence on the concentration of urine sugar. In the two experiments in which the rate of glucose injection was 1.8 grams per kilo body weight per hour, pituitrin administration was accompanied by a similar augmentation of glycosuria and a slightly higher concentration of urine sugar.

Determinations of plasma pH and CO_2 content of blood drawn before and at the end of the injection period were made in experiments on two animals (table 2). In one experiment with pituitrin no change in plasma pH was found. In another a fall of 0.05 pH was noted. In the two experiments in which glucose and pituitrin were administered the plasma CO_2 fell 10 volumes per cent as compared to a fall of 5 volumes per cent for the control experiments. We have frequently noted as great or greater changes than these in experiments with glucose injections, involving similar degrees of glycosuria and hyperglycemia.

The hemoglobin values at the end of the injection period in terms of percentage of the initial were as follows: (700 A), 103; (700E), 94: (700 F), 102; (702 A), 92; (702 E), 72; (702 G), 93; (703 A), 91; (703 D), 80; (703 I), 91; (710 A), 73; (710 B), 70; (711 A), 95; (711 B), 75.

TABLE 2

Blood plasma pH and CO_2 content determined before and at the end of the injection

EXPERIMENT	NATURE OF INJECTION	CO_2			pH		
		Before	End	Difference	Before	End	Difference
700 E	Glucose	44	41	−3	7.35	7.40	+0.05
700 F	Glucose and pituitrin	50	37	−13	7.41	7.36	−0.05
703 A	Glucose	51	46	−5	7.39	7.37	−0.02
703 D	Glucose	50	45	−5	7.40	7.39	−0.01
703 I	Glucose and pituitrin	57	49	−8	7.43	7.43	0.00

Note: CO_2 recorded in volumes per cent.

In experiments in which the glucose was injected at the rate of approximately 4 grams per kilogram of body weight per hour the hemoglobin content of venous blood drawn at the end of the period averaged 89 per cent of the preliminary value. When pituitrin was injected the average on the same animals was 95 per cent of the preliminary value. In experiments on two animals in which a 16 per cent solution of glucose was injected at the rate of 1.8 grams per kilogram of body weight per hour the average terminal hemoglobin value was 84 per cent of the preliminary value as compared to 72 per cent in the experiments with pituitrin. Attention is called to the fact that in the experiments with pituitrin and the larger amounts of glucose, the volume of fluid lost in the urine exceeded the intake; whereas in experiments with the smaller amounts of glucose the reverse was true.

A summary of the values obtained for R. Q. and heat production appears in table 3. It is apparent that pituitrin has been without appreciable effect on either the R. Q. or heat production during the injection period. The values obtained in the post-injection period would indicate a delayed return to preliminary values.

During the course of these experiments frequent observations were made of body temperature, pulse, and respiratory rate. In the experiments with glucose alone, a slight rise of body temperature was noted at the end

TABLE 3

Respiratory quotient and calories per square meter of body surface per hour

Based upon Rubner's constant in Meeh's formula $\sqrt{11.2 \text{ weight in grams}^2}$

EXPERIMENT	NATURE OF INJECTION	PRELIMINARY		INJECTION PERIOD		POST INJECTION	
		R.Q.	Calories	R.Q.	Calories	R.Q.	Calories
700 A	Glucose	0.81	39.90	1.00 (1) 0.93 (3)	57.20 (1) 61.60 (3)	0.85 (3)	42.30 (3)
700 E	Glucose	0.77	39.90	0.98 (1) 1.02 (3)	47.00 (1) 56.00 (3)	0.81 (5)	39.80 (5)
700 F	Glucose and pituitrin	0.79	32.10	0.92 (3)	59.80 (3)		
702 A	Glucose	0.78	42.10	1.00 (1) 0.98 (2)	68.50 (1) 52.40 (2)	0.85 (1) 0.82 (4)	49.00 (1) 40.50 (4)
702 E	Glucose	0.82	41.60	0.98 (1) 0.98 (2)	68.20 (1) 50.40 (2)	0.87 (2)	44.30 (2)
702 F	Glucose and pituitrin			1.08 (1)	56.70 (1)	0.96 (1) 0.89 (4)	52.40 (1) 43.40 (4)
702 G	Glucose and pituitrin	0.81	37.50	0.88 (1) 0.90 (2)	76.30 (1) 71.50 (2)	0.91 (2)	59.00 (2)
703 A	Glucose	0.80	37.60	0.96 (1) 0.97 (2)	56.80 (1) 61.80 (2)	0.83 (3)	35.10 (3)
703 D	Glucose	0.78	42.80	1.00 (1) 0.87 (2)	59.20 (1) 68.70 (2)	0.83 (3)	38.40 (3)
703 I	Glucose and pituitrin	0.83	37.40	1.04 (1) 0.86 (2)	47.60 (1) 56.90 (2)	0.85 (3)	46.10 (3)
Average...	Glucose	0.79	40.32	0.97	58.98	0.84	41.34
Average...	Glucose and pituitrin	0.81	35.67	0.95	61.47	0.905	50.23

Note: Figures in parentheses refer to the time in hours following the beginning and following the end of the injection.

of the injection, ranging from 0.5° Fahr. to 1.6° Fahr. and averaging 1° Fahr., with no change in respiratory rate and a slight acceleration of pulse

rate. In the experiments with glucose and pituitrin we observed either no change or a slight fall of body temperature. The pulse rate was usually slowed and respiration rate somewhat accelerated.

Discussion and conclusions. The experiments reported in this paper confirm the findings of Goetsch, Cushing and Jacobson (1911), Achard, Ribot and Binet (1919), and other investigators in that the administration of extracts of the pituitary gland resulted in a diminished capacity to handle carbohydrates. This was evidenced by the finding of a greater degree of glycosuria and hyperglycemia in the experiments in which pituitrin was injected simultaneously with the glucose. This effect was noted early in the course of the experiment and does not appear to be more marked at the end of the injection period. This finding may indicate that pituitrin, in these doses, exerted at once its maximum effect on the factors responsible for the removal of glucose from the blood and that further administration did not augment this effect due to its rapid elimination, destruction or counter action by other mechanisms.

The fact that the concentration of glucose in the urine was approximately the same with pituitrin administration as without and that the amount of glucose lost in the urine was comparable to that found in other experiments involving similar degrees of hyperglycemia, leads us to believe that a pituitrin action on the kidney has not been an important factor in these experiments.

When glucose was injected at the rate of 4 grams per kilo body weight per hour in normal dogs by the continuous intravenous method, the response, as judged by amount of sugar retained, blood sugar values, R. Q. and heat production, was quite constant for any one animal to repeated injections (Boyd, Hines and Leese, 1925). We[2] have found that practically all of the glucose injected can be accounted for by elimination in the urine, oxidation or conversion into glycogen in the tissues. On the basis of tissue analysis for total sugar and glycogen content before the injection and in the post-injection period when the blood sugar had returned to the preliminary value, we have calculated that the increased glycogen content of the liver accounts for about 40 per cent of the stored sugar and the muscles approximately 60 per cent.

The values obtained for the R.Q. and heat production indicated that pituitrin administration has been without appreciable effect on the quantity of glucose disposed of by oxidation. The R.Q. approximated unity during the time glucose was being administered. This would seem to indicate predominant glucose oxidation and gives no positive evidence of fat formation. This finding is interesting in view of the experiments reported by Coope and Chamberlain (1925) in which pituitrin injections resulted

[2] Unpublished experiments.

in a marked increase in the fat content of the liver of rabbits. However these investigators employed a different experimental animal and much larger doses of pituitrin.

The increased heat production occurring during the period of glucose injection was not appreciably modified by pituitrin administration. We believe that the extra heat production may not be due entirely to the stimulating effect of carbohydrate plethora. Our preliminary values, in spite of diet control and accustoming the animal to the routine of the experiment, are higher than those accepted as basal by Lusk and Du Bois (1924), Kunde and Steinhaus (1926) and others, although well within the range reported by Boothby and Sandiford (1923). This we think can be accounted for by the increased tension and tonus caused by the unavoidable handling of the animal. Such factors may be enhanced during the course of the experiment. We are however justified in concluding that pituitrin has not affected the amount of glucose disposed of by oxidation.

The somewhat delayed return of the blood sugar, R.Q. and heat production to the preliminary values in the post injection period may not be due to any direct action of the pituitrin per se. We have noted a similar delay in the return to preliminary values in experiments in which larger quantities of glucose were injected or in which an equivalent amount of glucose was lost in the urine due to altered conditions which did not involve pituitrin administration.

The administration of Ringer's solution at the rate of 15 or 25 cc. per kilo per hour was without effect on the blood sugar level. However when pituitrin in amounts equal to those employed in the glucose experiments was added to the Ringer's solution a small but definite increase in blood sugar was found in every experiment. This effect was noted early in the course of the injection and does not progressively increase. The conditions of our experiments do not permit us to conclude whether this should be interpreted as being due to a specific stimulation of glycogenolysis, altered distribution of glucose between cells and plasma, or to the necessity of a higher level of blood sugar to adequately supply the cells with glucose under the possible conditions of an altered permeability and area of the capillary bed.

It was apparent that the effect of pituitrin injection has been to cause a decreased rate of removal of glucose from the blood by the tissues. This resulted in a greater degree of hyperglycemia and glycosuria. The amount of glucose disposed of by oxidation was not appreciably altered. Hence the amount stored in the tissues must be less. There are several factors which are known to modify the rate of glucose removal from the blood by the tissues, such as the acid base balance of the blood, the supply of the pancreatic hormone, the condition of the circulation in the various tissues, and apparently the quantity of adrenalin discharged into the

circulating blood stream. Burn (1923) has reported experiments showing that pituitrin administration is apparently antagonistic to the usual blood sugar changes produced by adrenalin and insulin. Krogh (1922) has demonstrated that pituitrin is capable of causing a constriction of the capillaries and believes that a function of this hormone is to regulate capillary tonus. The recent experiments of Cajori, Crouter and Femberton (1925) demonstrate the marked effect of circulatory changes produced by hydrostatic conditions upon the response to ingested glucose. The limited number of observations made on the acid base balance of the blood suggest that the effect of pituitrin administration upon carbohydrate metabolism in these experiments was not due to changes in blood reaction. However these findings do not exclude the possibility of local acid base changes in the tissues. Although we do not know what the effect of pituitrin in these doses may be upon the capillaries of such tissues as liver, muscle and pancreas the slowing of the pulse observed is suggestive of an increased peripheral resistance. We believe that it will be fallacious to attempt to explain the apparent antagonism between pituitrin and insulin or adrenalin until due consideration is given to a possible altered tonus and permeability of the capillary bed. When the circulatory factor has been evaluated, the mechanism of pituitrin action on carbohydrate metabolism may not be so obscure.

SUMMARY

1. The administration of pituitrin in doses of approximately 0.05 cc. per kilo body weight per hour together with glucose by the continuous intravenous method results in a decreased retention of the injected sugar by the tissues of the dog. This is evidenced by a greater degree of hyperglycemia and glycosuria than in control experiments on the same animals with glucose alone.

2. Pituitrin administration is without appreciable effect on the respiratory quotient and extra heat produced during the glucose injections.

3. The effect of pituitrin is apparently not due to acid-base changes in the blood.

4. A pituitrin action on the kidney has not been an appreciable factor in these experiments.

5. The evidence for decreased retention of glucose resulting from pituitrin administration appears early in the course of the experiment and does not appear to be more pronounced at the end of the period.

6. It is suggested that an altered capillary circulation may be an important factor in the mechanism of pituitrin action.

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DETERMINATION OF A FORMULA FOR THE SURFACE AREA OF THE DOG TOGETHER WITH A CONSIDERATION OF FORMULAE AVAILABLE FOR OTHER SPECIES

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Students of metabolism are well aware of the importance of the surface area of the body as a unit to which to relate the heat production of warm-blooded organisms. In their review of the literature concerning basal metabolism, Boothby and Sandiford (1924) make the following significant statement: ". . . . . It is considered that the surface area is the most exact of any biometric measurement yet suggested which will indicate the probable relative values of an active protoplasmic mass in different individuals with approximate accuracy."

Fairly extensive measurements of the surface area of human beings and other species have been made. A formula relating the surface to volume, namely: Surface = K volume$^{2/3}$ was developed by Meeh (1879). If the specific gravity is assumed to be constant, weight may be substituted for volume in this expression. Rubner (1883) generalized concerning surface in relation to heat production by formulating his so-called *surface area law*. The value of K in the above formula was found to vary with different species depending probably upon the *pattern* shape for the species. That this expression is only roughly approximate has been shown by numerous investigators[2] who have evaluated K for different species. The deviations from the surface as predicted by this formula have been correlated quite definitely with the variations in shape associated with the state of nutrition of the individual, whether thin or obese.

With the development of highly accurate methods for the measurement of heat output, and the desire to study the basal metabolism in disease, where often the shape of the individual has become markedly altered, it became necessary to estimate the surface area with greater accuracy than the Meeh-Rubner or similar formulae possessed. Du Bois in his mono-

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[2] Pfaundler (1916) has prepared a table showing the values of the Meeh-Rubner "constant" for different species together with fairly complete citations of the literature involved. See table VIII, page 63 et seq., in Pfaundler's paper.

graph on *Basal Metabolism in Health and Disease* has pointed out the absurdity of measuring the heat production with an error of approximately 2 per cent and then expressing the metabolism in terms of units of surface area which is estimated by a formula that may be inaccurate to the extent of 10 to 30 per cent. In an attempt to meet the difficulty, Du Bois and Du Bois (1915) made a series of careful measurements of the surface area of human beings of different shapes and states of nutrition and developed the well-known linear and height-weight formulae which have been used widely in clinical calorimetry. The series of types measured and the validity of these new expressions were extended by the work of Sawyer, Stone and Du Bois (1916).

The dog has been used extensively for experimental work in metabolism. Lusk and his students have made numerous studies of the heat production of dogs under various conditions.[3] Although the basal metabolism of these animals was measured with a highly accurate calorimeter, the results were expressed usually in terms of calories per unit of surface area, the latter being estimated by the Meeh-Rubner formula, namely, $S_{\text{sq. cm.}} = 11.2\ W_{\text{gm.}}^{2/3}$. All students of the basal metabolism of the dog, so far as we have been able to ascertain, have been forced to estimate surface by the Meeh-Rubner expression. A test of the accuracy of this formula for the dog by some such technic as that employed by Du Bois and Du Bois (1915) in their measurements of surface area of human beings, does not appear to have been made.

The advantage of having as highly accurate formulae as possible for estimating the surface area of dogs hardly needs extended argument. These considerations together with the fact that the writers have been engaged on problems of metabolism with the dog in which more accurate estimations of surface area were desired, led to the work reported in this paper.

Earlier attempts at derivation of formulae. The Meeh-Rubner formula expresses surface area purely as an exponential function of weight. In this connection Krogh (1916)[4] stated that the "line of inquiry initiated by Dreyer is much more likely ultimately to clear up the relationship between size and metabolism. The metabolism should not therefore be expressed per square meter or any other unit of surface but as a function of W^n. For warm-blooded animals n can be taken, at least provisionally, as $\frac{2}{3}$." It is not our desire to review the debate concerning the significance, of surface area as a unit with which to express basal metabolism, as the above quotation from Krogh might imply. However, in attempting to develop a new formula for estimating surface area it seemed worth while

[3] Many of these studies are reported in a series of papers published in the Journal of Biological Chemistry. See Volume vii et seq.

[4] See page 140.

to follow the suggestion of Krogh and to consider the work of Dreyer and his associates. Dreyer and Ray (1909–10) and Dreyer, Ray and Walker (1912–13) made numerous measurements of the cross section of the aorta, cross section of the trachea and blood volume in various animals and found that the results could be expressed in a formula very similar to the Meeh-Rubner expression for surface, namely, $A = KW^n$ where A is the aorta cross section (or other measurement in question), K is a constant whose value varies with species, W is the body-weight, and n is approximately 0.70 to 0.72 instead of 0.667, which would be the two-thirds power. These investigators made no measurements of body-surface so far as the writers

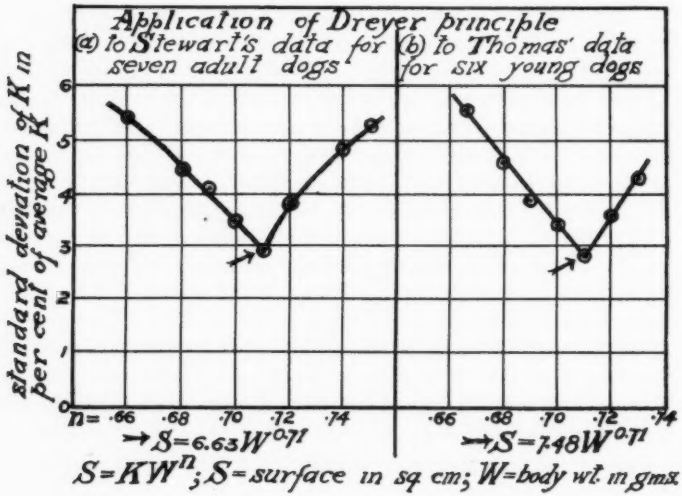

Fig. 1

have been able to determine, but assumed that these various biotic measurements were functions of surface because their formulae were so similar to the Meeh-Rubner expression for surface. Likewise Dreyer (1919) developed many interesting formulae in his studies of vital capacity, and theorized concerning the relationship of vital capacity to body-surface.

As a first attempt toward developing a new formula for estimating the surface area of dogs, one of us (C) several years ago used data of Stewart (1921–22) on this species to test Dreyer's principle by calculating the value of K after assigning arbitrary values to n. The exponent was varied from 0.667 through 0.68, 0.69, etc., up to and including 0.75. Best agreement of the values for K was obtained when n was 0.71, the standard deviation of the K values from the mean being $\pm$ 2.9 per cent. When the

two-thirds power was used, the standard deviation of K proved to be ± 5.4 per cent of the mean. The formula thus developed, using only the data for the seven dogs described by Stewart as being "normal," was as follows:

$$\text{Surface}_{\text{sq.cm.}} = 6.63\ \text{weight}^{0.71}_{\text{gm.}} \quad (1)$$

The abnormal cases were not considered here because this expression gives surface simply as a function of body-weight, and Du Bois and Du Bois (1915) have pointed out that whenever surface is estimated on the basis of weight alone, a large error is involved in those cases that differ to any great degree from the "normal" pattern shape of the species, i.e., cases of great emaciation or obesity.

The data of Thomas (1911), who measured the surface areas of six young dogs, were similarly treated and found to yield the following expression:

$$\text{Surface}_{\text{sq.cm.}} = 7.48\ \text{weight}^{0.71}_{\text{gm.}} \quad (2)$$

It will be noticed that the two expressions thus obtained differ only in the values of the constant, K; the values for the exponent of the weight are identical not only with each other but with those in Dreyer's formulae for aorta cross section, etc.

It seems worth while to point out that the Meeh-Rubner expression has a more satisfactory theoretical and mathematical basis than that of Dreyer, in that the value of the exponent, namely, 2/3, is obtained from consideration of the facts that surfaces of similar solids vary as the square whereas the volumes vary as the cube of the unit dimension. No good theoretical and mathematical basis has as yet been discovered by which to explain the strange facts that Dreyer has found, and which we have confirmed with the data from Stewart and Thomas, namely, that the exponent of the weight should be 0.71 instead of 0.667 which would be the two-thirds power. It is possible that this difference is due merely to the difference in accuracy of the methods used in obtaining the data. The new expressions therefore must be regarded as purely empirical ones best fitting the data from which they were developed.

The formulae presented above were based upon measurements of the area of skins of dogs; they give definitely lower values for the body surface than does the Meeh-Rubner expression. Uncertainty as to whether the methods adopted by Stewart and Thomas are the best for measuring surface area, and the desire to settle upon some more accurate formula, if possible, impelled us to undertake the task of actually measuring the surface area of many dogs.

EXPERIMENT. *Method.* The methods that have been used for measuring body-surface area have been reviewed by Du Bois and Du Bois (1915)

and therefore need not be given here.[5] The one we considered most accurate and chose to follow was essentially that employed by the above investigators in their measurements of the surface area of human beings. The animal was covered with a single layer of gauze over which a paper mold was tightly plastered; the paper model was then removed, cut into as many pieces as necessary to lie flat, the pieces printed on photographic proof paper, the printed pieces then cut out and weighed upon a delicate balance. The weights of known areas of the same paper supplied data for calculating the factor by which to convert the printed weighed model to units of area. Figures 2 and 3 are photographs of such a model.

Measurements made. Seven dogs ranging in body weight from 3.39 to 32.64 kilograms body weight were thus measured. One model was made using an animal anesthetized by amytal; the other dogs were dead when

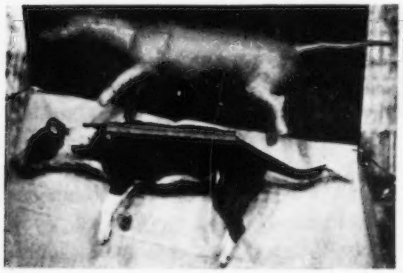

Fig. 2

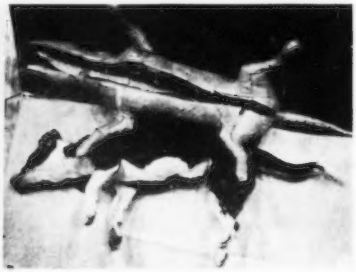

Fig. 3

Fig. 2. Photograph of a dead dog and its model, showing the molding and the "fine fit."

Fig. 3. Photograph showing the method of removing the model, which is very thin and yet firm.

the molds were made. Certain parts of the body could not be covered accurately. The ears were not covered but were carefully traced out on paper, this pattern traced twice for each ear on opaque paper and these final tracings cut out and printed along with the corresponding model. The same procedure was followed for estimating the surface between the spread out toes and any other area—soft vulva tissue, for example—that seemed unusually difficult to mold accurately. No attempt was made to include the inner recesses of the external ear in the total surface area.

The linear formula of Du Bois is apparently the most accurate expression available for estimating the surface area of human beings. The principle here used is that area is given by the product of length and breadth; for

[5] Very recently Brody and Elting (1926) described an ingenious instrument by which surface area may be estimated on the living subject.

its application to the human being nineteen separate measurements of what might be regarded as average lengths and breadths of parts of the body are necessary. We made no attempt to develop a similar expression for dogs but contented ourselves with securing data which would allow the construction of simpler formulae of the height-weight or other types. We measured the length of each animal or what might be regarded as analogous to von Pirquet's (1917) "sitting height" or Dreyer's "stem length," taking for this purpose the distance from the tip of the nose to the anus. As an aid in visualizing the nutritive state of the animal, and, we hoped, in giving another datum with which to estimate surface area, the circumference of the chest at the plane of the first nipple was measured. Certain other measurements were made in some animals which, it was hoped, would enable one to correct for surface area as influenced by the state of nutrition. Skeletal measurements obviously are little if at all affected by the nutritional state and may therefore be called *constant*, in contrast

TABLE 1

DOG NUMBER	4	6	1	7	2	5	3
Body weight grams	3,390	5,350	5,450	10,150	17,250	25,930	32,640
Length from nose to anus, cm	51	62	74	76	98	100	103
Circumference of chest, cm	35	37.5	36	49	56	67	75
$\frac{\text{Weight}^{\frac{1}{3}}}{\text{Length}} = N_{obs.}$	0.295	0.281	0.260	0.285	0.264	0.296	0.310
Surface area, sq. cm	2,320	3,284*	3,815	5,070†	8,104	9,106‡	10,763

* Average of two printings of same model; d = 1.2 per cent of average.
† Average of two models differing by 2.66 per cent.
‡ Model made on living dog anesthetized by amytal.

to such measurements as the circumference of the abdomen, for example, which measurement might therefore be called *nutritional*. None of these data proved useful, however, in arriving at a formula for surface area.

The principle upon which von Pirquet (1917) based his *pelidisi* was found useful, however, in affording an index of the state of nutrition. We have adopted $N_{obs.}$ as a symbol for this index. It signifies the ratio of the cube root of the body weight in grams to the body length in centimeters, length being defined as above. This can be remembered as indicating the *nutritive state observed* in the individual case in question.

The varieties of shape of the nose occurring in the dog, and the variations noticed when repeated measurements of length were made by different individuals on the same dog were the chief reasons for giving length measurements to the nearest centimeter.

Table 1 gives the important data obtained from all of the dogs studied.

Error of the method. Sawyer, Stone and Du Bois (1916) tested their

technic by making a paper mold of a bowling ball. When one layer of gauze was used over the ball and fitted very tightly, the method showed practically no error. A looser fit gave an error of about + 3 per cent.

We made two models of each of two bowling balls of 12.05 and 21.6 cm. average diameter respectively, and took special pains to fit the molds tightly. The average agreement of two similar models with each other was 1.3 per cent. Every model, however, gave a result several per cent

TABLE 2

A comparison of formulae for estimating surface area of dogs

DOG NO.	SURFACE AREA	$S = 6.67\, W^{0.70} \times \sqrt[0.34]{N_{obs.}}$ (1)*		$S = 3.94\, W^{0.42} \times L^{0.76}$ (2)		$S = 4.381\, W^{0.425} \times L^{0.725}$ (3)		$S = 1.365 \left[\frac{2W}{L} + 4L\right] \sqrt{\frac{W}{L}}$ (4)		$S = 11.2\, W^{\frac{2}{3}}$ (5)	
	S measured	S calculated	d from measured S	S calculated	d from measured S	S calculated	d from measured S	S calculated	d from measured S	S calculated	d from measured S
	sq. cm.	*sq. cm.*	*per cent*	*sq. cm.*	*per cent*	*sq. cm.*	*per cent*	*sq. cm.*	*per cent*	*sq. cm.*	*per cent*
4	2,320	2,278	−1.81	2,376	+2.41	2,398	+3.36	2,451	+5.65	2,538	+9.40
6	3,284	3,284	0	3,355	+1.54	3,354	+2.13	3,381	+2.33	3,426	+3.69
1	3,815	3,934	+3.12	3,848	+0.87	3,843	+0.73	3,667	−3.88	3,484	−8.68
7	5,070	5,075	+0.09	5,101	+0.60	5,102	+0.63	5,160	+1.78	5,251	+3.40
2	8,104	7,948	−1.93	7,730	−4.62	7,686	−5.16	7,579	−6.48	7,476	−7.75
5	9,106	9,418	+3.47	9,317	+2.21	9,278	+1.89	9,303	+4.36	9,810	+7.73
3	10,763	10,550	−1.98	10,500	−2.44	10,450	−3.04	10,870	+0.99	11,440	+6.29
Average.....			±1.77		±2.10		±2.42		±3.64		±6.70

* This formula can also be expressed as follows: $S = 2.268\, W^{0.367}\, L$. The first expression has this advantage, however, that its character as a combination of Meeh-Rubner-Dreyer ideas with a correction for the nutritive state of the subject is at once apparent.

higher than the surface as estimated by geometry. The average plus error for the four measurements was 3.67 per cent.

In fitting the model tightly to the body there is some folding of the skin particularly in the region of the shoulders and neck, which means that some surface escapes accurate covering by the mold. Inasmuch as this error is almost impossible to evaluate but is opposite in sign to the inherent positive error of this method in our hands, it seemed advisable not to make any arbitrary correction of the figures obtained.

The error in printing the same model twice was of the order of 1.2 per cent. In the case of dog 7, *two entirely separate molds were made;* they yielded surfaces differing by ±1.34 per cent of the mean.

Formulae derived. A comparison of formulae is given in table 2. The first formula in the table seems new in type; the second is similar in principle to the Du Bois height-weight expression; the third is the Du Bois formula with a different value for the equating constant; the fourth is based on Bardeen's (1920) idea of regarding the body as an elongated block; while the fifth is that developed by Meeh and Rubner. The surface area values yielded by the formulae are given and comparison with the measured value is easily made by inspection.

Formula 1 is essentially the Meeh-Rubner expression multiplied by a factor correcting for the nutritive state of the subject. The principle upon which this factor is based is the same as that employed by von Pirquet (1917) in arriving at his *pelidisi*. For unit increase in length (sitting height, or stem length) there is unit expansion in the three dimensions which determine volume. If specific gravity is assumed to be constant, weight instead of volume may be taken as the factor with which to compare length. Inasmuch as weight is a function of three dimensions, the cube root of weight is the proper unit with which to compare unit change in length. It is obvious that the value of the ratio $\frac{W^{1/3}}{L}$ should be practically constant in subjects of the same species of approximately the same age[6] and characterized by the same nutritive states. It is also obvious that the values for this ratio will be higher in obese individuals than in thin subjects.

There is less surface per unit of volume in a sphere than in any other shaped solid. Similarly, the more obese an individual is, the less surface he has per unit of weight. If the maximum degree of expansion of the three dimensions that determine weight is determined for the species by evaluating the *nutritive index* in the most obese individuals obtainable, one secures a reference or standard or maximum value for this ratio with which to compare the value for the individual in question. It is suggested that this species maximum or "standard" be designated by the symbol N_m, and the value observed in the case in question $N_{obs.}$ We have chosen to designate the ratio $\frac{N_m}{N_{obs.}}$ as the *nutritive correction factor* and use it to

[6] Subjects differing widely in age show large differences in water content (Moulton, 1923). It is possible that similar differences in specific gravity exist. This might account in part for the failure of surface area formulae based on consideration of weight alone and found satisfactory for adult organisms, to hold for the very young individuals.

correct the surface as given by an expression of the Meeh-Rubner type for the nutritive state of the subject.

In applying these ideas to our data for the dog, the maximum value of N for this species was found to be approximately 0.34, and this value was therefore selected as N_m. Greatly emaciated dogs were found to have a value as low as 0.26, whereas the values for dogs in a good average state of nutrition were between 0.29 and 0.31.[7]

With the value for N_m thus fixed, calculations were made to determine what value for the exponent of the weight gave best agreement with the data. This agreement was studied by comparing the values of K. Best agreement was given by the value 0.70. The average value for K was 6.67 with a standard deviation from this average of ± 2.27 per cent; the ratio $\frac{\text{standard deviation}}{\text{average deviation}}$ was equal to 1.10.

It will be noticed that length enters the formula as a datum of the nature of a maximum diameter. In discussing his height-weight formula for humans, Du Bois does not give any opinion as to the significance of height, but treats it as a uni-dimensional datum easy to obtain accurately, and uses with the tri-dimensional datum weight to develop a bi-dimensional expression for surface.

Formula 1 as given in table 2 can be simplified to yield an expression of the Du Bois type, namely,

$$S = 2.268\ W^{0.367} \times L. \tag{3}$$

We prefer to write the formula as stated in the table rather than in this simplified form, because of this advantage; its character as a combination of Meeh-Rubner-Dreyer ideas with a correction factor for the nutritive state of the subject is thus made apparent. If the simplified form is examined closely, its empirical nature becomes clear. The expression is not perfectly bi-dimentional on both sides of the equality sign. Those of our readers who do not care to accept the theory involved in the development of formula 1, may thus employ the simplified form and regard the formula as a purely empirical expression of the length-weight type applicable to the dog. It is certainly significant, however, as inspection of table 2 shows, that formula 1 gives decidedly the best agreement with the data of all the formulae developed, *and was arrived at by following the line of reasoning indicated above.*

Various charts have been made for use in quickly estimating the surface area of human beings by Du Bois' formula. Boothby and Sandiford (1921)

[7] The animals giving values between 0.29 and 0.31 were in that state of nutrition which they determined for themselves, when allowed all they wished of a "perfect" ration (Cowgill, 1926).

prepared a three parallel scale nomogram for this purpose. A similar nomogram for the dog based on the simplified form of formula 1 may be constructed, as well as one of a new type based on the unsimplified formula 1. The latter allows one not only to estimate surface area but to ascertain the state of nutrition of the animal, and is shown in figure 4.

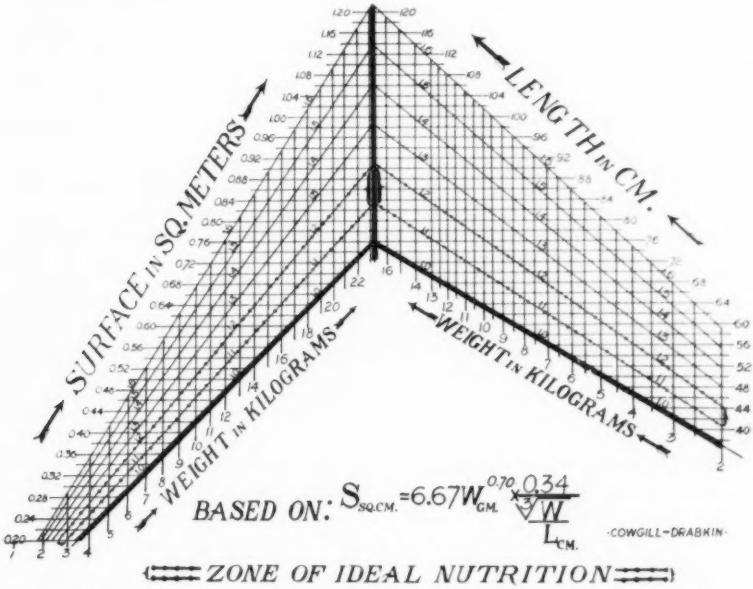

Fig. 4. Chart for estimating the state of nutrition of the dog and the body surface area when given the length and the body weight. Directions for use are given in the text.

This nomogram is constructed as follows. A plot of the expression $\frac{W^{1/3}}{L} = 0.34$ is made with length on the ordinate axis and $W^{1/3}$ as abscissa. The points for $W^{1/3}$ are labelled with the corresponding body weight in kilograms. The 45° diagonal running through the origin corresponds to the slant 0.34 which is the value of N_m for the dog. This diagonal is also the locus of points for $\frac{N_m}{N_{obs.}} = 1$. Other diagonals corresponding to values for 1.1, 1.2, etc., up to and including 1.6 are then drawn. Values of $N_{obs.}$ between 0.29 and 0.31 (those given by animals in an excellent state of nutrition) give values of $\frac{N_m}{N_{obs.}} = \frac{0.34}{0.29} \ldots \frac{0.34}{0.31}$ or from 1.1 to 1.2 approximately. Points for length and weight that fall between these diagonals therefore may be regarded as indicating an excellent state of nutrition. Points above the 1.2 diagonal indicate thinness, whereas points below the 1.1 diagonal denote some degree of obesity.

Suppose our above plot has been made on thin paper. By turning the paper upside down and holding it to the light so as to be able to see the lines drawn on the other side, one obtains a chart differing from the above only in this respect, that the scales read from right to left instead of the more usual plan of left to right.

Consider now the left hand half of the nomogram. A plot of the expression $S_{\text{sq.cm.}} = 6.67\ W_{\text{gm.}}^{0.70}$ is made with S as ordinate and $6.67\ W^{0.70}$ as abscissa. The 45° diagonal running through the origin is the locus of all points for the expression $S = 6.67\ W^{0.70}\ \frac{N_m}{N_{obs.}}$ where the nutritive correction factor $\frac{N_m}{N_{obs.}} = 1$. Additional diagonals are drawn corresponding to the above expression but with $\frac{N_m}{N_{obs.}} = 1.1$, 1.2 etc., up to and including 1.6. The two plots may then be combined as indicated in figure 4.

The nomogram is used as follows. Using the right hand half, length and weight are read and the point of intersection then placed as accurately as possible with reference to the diagonals. This latter reading is made on a line approximately perpendicular to the diagonals. This reading also gives the value of the *nutritive correction factor*. The pencil is moved from the nutritive correction factor point in the zone between diagonals up and across the middle of the chart, down to the left keeping between the corresponding diagonals. A position with reference to the diagonals on the left corresponding to the point for the nutritive correction factor on the right is located which intersects the reading for weight on the left. Surface is now read at the left. If reasonable care is taken in locating points with reference to the diagonals, this chart will give surface accurately to three significant figures, which is all that is desired.

Formula 2 was developed by applying the height-weight principle in a purely empirical fashion. This expression is not absolutely bi-dimensional, but is very nearly so.

It was not found possible to improve the agreement by changing the values of the exponents of the weight and length *at the same time keeping the formula perfectly bi-dimensional in character*. Formula 3 was the best that could be found to fit these conditions. This expression differs from the Du Bois formula for human beings in only two respects, namely, length—tip of nose to anus—is used instead of height, and the value of the equating constant is different.

Formula 4, the principle of which was developed by Bardeen (1920), proves to be less accurate than formulae 1, 2 and 3, but somewhat more reliable than the Meeh-Rubner expression.

Considering the way the Meeh-Rubner formula was obtained, it is surprising to find that it gives as good agreement as it does. This may perhaps be explained by the assumption that our series of animals did not include sufficiently extreme types. We intend to measure the dachshund and the greyhound when the opportunity is presented.

Significance of new expression for different species: Dogs. The probable significance of the new formula 1 for determining the surface area of dogs is well illustrated by consideration of the data for basal metabolism of dogs

and man published by Lusk and Du Bois (1924). The average figure for dogs published more recently by Kunde and Steinhaus (1926) is the same. Using the Meeh-Rubner formula for estimating surface area, the average number of calories per day per square meter for female dogs is 772. According to the series of one hundred and three individuals listed in table D of the monograph by Harris and Benedict (1919), the average basal metabolism of women, with which these female dogs might more properly be compared, is 850 calories per hour per square meter with a standard

TABLE 3

Basal metabolism of the dog

Dog	Date	Body weight	Surface*	Calories				
				Per hour	Per day	Per hour per sq. m.	Per day per sq. m.	d from average
		kgm.	*sq. m.*					*per cent*
XIX† Length 68 cm.	1920	9.9	0.450	17.6	422	39.08	938	+12.2
	1921	9.4	0.440	17.5	420	39.74	954	+14.1
	1923	11.5	0.475	16.5	396	34.70	833	+0.4
	1924	11.5	0.475	16.5	396	34.70	833	+0.4
XXVII‡ Length 68.2 cm.	1926							
	April 14	6.30	0.382	12.57	302	32.90	790	−5.5
	April 15	6.35	0.383	12.56	301	32.76	786	−6.0
	April 19	6.50	0.387	12.73	306	32.91	790	−5.5
	April 21	6.60	0.389	12.94	311	33.28	799	−4.4
	April 26	6.80	0.393	12.99	312	33.05	793	−5.1
	April 28	7.00	0.397	13.37	320	33.58	806	−3.6
	May 5	7.00	0.397	13.96	335	35.13	843	+0.8
	May 7	7.00	0.397	14.33	344	36.06	865	+3.5
Average							836	±5.1

* $S = 6.67\ W^{0.70}\ \frac{0.34}{N_{obs.}}$

† Lusk, G. and E. F. Du Bois. 1924. Journ. Physiol., lix, 213.

‡ Personal communication from G. Lusk.

deviation ± 9.1 per cent of the average. When our new formula 1 of table 2 is used to estimate the surface of dog XIX, which is the only animal in the series (reported by Lusk and Du Bois) whose length is known, the data for this animal give 890 calories per square meter per day as the average value. These data for dog XIX are included in table 3.

Prof. Graham Lusk has kindly furnished us with additional determinations of the basal metabolism of the dog, namely, those made quite recently on animal XXVII of his series. We have made calculations for this dog similar to those just described for dog XIX. The data are incorporated in table 3.

A preliminary comparison of the basal metabolism of these female dogs with women is given in table 4. The agreement is remarkable.

Harris and Benedict (1919) in their monograph devoted a long chapter to *A Critique of the Body Surface Law*. They limited their study of its applicability "to variations within the human species, in short to its intra-specific and not its inter-specific applicability."[8] They admit, however, that "it has furnished a somewhat better basis for the prediction of the metabolism of an unmeasured subject than does body weight." With reference to their objections 1, to the idea that the surface functions as an agent for body cooling, and 2, that the surface area law does not apply to cases of inanition, etc., the work reported in this paper contributes nothing. It does seem worthwhile to point out, however, that their remaining objection seems to be based on a study of the variations of basal metabolism within one species, namely, man. Final rejection,

TABLE 4

A comparison of the basal metabolic rates of the dog and man

	FEMALE DOGS*	WOMEN†
Calories per square meter per day—average	836	850
Standard deviation—calories	55	87
Standard deviation in per cent of average	6.6%	9.1%
Standard deviation / Average deviation	1.28	1.48
Probable error of standard deviation—calories	11	5.8

* See table 3 of this paper.

† Harris, A. and F. G. Benedict. 1919. See table D, pp. 44–47.

however, should await a consideration of comparable data from different species. The formulae presented in this paper enable at least a preliminary comparison of the human being and the dog to be made with the results just presented. The average values for the two species, while not identical, are so nearly the same that, when the standard deviations for the two series are compared, the difference is not significant.

Inasmuch as the combination of ideas set forth in formula 1, table 2, has some novel features, it becomes of interest to determine the general significance and applicability of such an expression. In view of the fact that this formula may be simplified to a length-weight expression, a study of its validity for other species becomes essentially a study of the generality of a length-weight expression, viz., $S = KW^a \times L$.

Adult human beings. The papers of Du Bois and Du Bois (1915) and

[8] See page 199.

Sawyer, Stone and Du Bois (1916), and more recently that by Takahira (1925) are the only ones we have been able to find, which contain data on surface area together with other measurements from which one can accurately estimate a body length at all comparable to the length as defined in formula 1, table 2, for the dog. Calculations soon showed that height does not serve as well as the stem length or sitting height in such an expression as formula 1, table 2.

TABLE 5

DATA OF DU BOIS ET AL.				CALCULATIONS				
					$S = K_1 W^{0.71} N_{obs.}^{-0.63}$		$S = K_2 W^{0.425} Ht^{0.725}$ Average K_2† = 71.84	
Subject	Body weight W	Surface area S	Length $Ht-(O+R)$* L	$\frac{W^{\frac{1}{3}}}{L}$ $N_{obs.}$	K_1	d from average K_1	K_2	d from average K_2
	grams	*sq. cm.*	*cm.*			*per cent*		*per cent*
Benny L. . . .	24,200	8,473	54.6	0.530	5.500	+2.15	72.30	+0.64
Morris S. . . .	64,000	16,720	80.9	0.494	5.077	−5.70	70.65	−1.65
R. H. H.	64,080	18,375	84.0	0.476	5.371	−0.24	73.22	+1.92
E. F. D. B. . .	74,050	19,000	83.5	0.503	5.291	−1.73	70.85	−1.38
Mrs. McK. . .	93,000	18,592	72.9	0.621	5.442	+1.08	71.70	−0.20
Anna M.	6,270	3,699	41.2	0.448	5.291	−1.73	75.54	+5.15
Gerald S. . . .	45,250	14,901	78.8	0.452	5.294	−1.68	70.36	−2.06
Emma W. . . .	57,620	16,451	73.8	0.523	5.695	+5.78	72.56	+1.00
R. H. S.	63,000	17,981	80.8	0.492	5.500	+2.15	70.58	−1.75
Average					5.384	±2.47	72.21	±1.93

* The sum of measurements O and R (Du Bois) equals the distance from the symphysis pubis to the plantar surface of the foot.

† These values for K_2 and d are taken from the papers of Du Bois et al. The unit of weight for these calculations is the kilogram. The average K used in calculating percentage deviations is that obtained by Du Bois when he combined the above series of *measured* surfaces with a larger series of cases where surfaces were estimated by the linear formula. Du Bois regards the value 71.84 as the best one to use.

Table 5 presents the data given in the first two papers just cited, together with a comparison of a new formula of type I, table 2, for human beings, with the Du Bois height-weight expression. The value of N_m for humans was selected as 0.63, this figure being only slightly larger than that of $N_{obs.}$ for the case "Mrs. McK." who was extremely obese. The exponent of the weight in this formula is 0.71 as compared with 0.70 in the formula for the dog. The average deviation is ±2.47 per cent of the mean, whereas the Du Bois height-weight formula shows ±1.93 per cent of the average.

The data of Takahira were obtained from ten adult Japanese subjects. We are indebted to Dr. K. Sugimoto, of our laboratory, for translating such material from Takahira's paper as is pertinent to this work. A

formula of type 1, table 2, was constructed from these data. As shown in table 6, this expression allows one to calculate the surface with an accuracy of ±1.98 per cent. Inspection of the figures for $N_{obs.}$ in table 6 shows that the group of individuals studied could not have been characterized by the extremes of shape that were included in the Du Bois series. This is emphasized by the remarkable agreements shown by the values of the constants for the several formulae compared, especially those of Takahira and of Du Bois.

The value for N_m, 0.56 was arrived at by assuming the average value of $N_{obs.}$, namely, 0.45, to be capable of increase according to the ratio of the

TABLE 6

	DATA FROM TAKAHIRA, 1925					CALCULATIONS						
							Cowgill and Drabkin $S = K_1 W^{0.60} \cdot \frac{0.66}{N_{obs.}}$			Du Bois and Du Bois $S = K_2 W^{0.425} \cdot Ht^{0.725}$		Takahira $S = K_3 W^{0.427} \cdot Ht^{0.718}$	
Subject	Surface area	Body weight	Height	$O + R$*	Stem length $Ht - (O+R)$	$\frac{W^{\frac{1}{3}}}{L}$							
	S	W	Ht		L	$N_{obs.}$	K_1	d from average K_1	K_2	d from average K_2	K_3	d from average K_3
	sq. cm.	*grams*	*cm.*	*cm.*	*cm.*			*per cent*		*per cent*		*per cent*
1	15,923	51,000	169.60	84.75	84.85	0.437	18.58	+0.76	3.846	0	3.898	0
2	14,964	49,500	160.15	75.50	84.65	0.437	17.81	−3.42	3.816	−0.75	3.869	−0.74
3	14,913	48,000	162.60	79.50	83.10	0.437	18.08	−1.95	3.812	−0.86	3.874	−0.62
4	14,647	50,100	151.80	74.00	77.80	0.474	18.78	+1.84	3.864	+0.49	3.916	+0.44
5	16,010	55,600	163.00	79.00	84.00	0.454	18.46	+0.11	3.837	−0.21	3.890	−0.21
6	14,593	46,300	156.5	79.50	77.00	0.466	19.28	+4.56	3.893	+1.25	3.926	+0.72
7	15,621	55,900	161.50	77.50	84.00	0.455	17.99	−2.44	3.760	−2.21	3.813	−2.18
8	15,425	51,200	156.20	76.00	80.20	0.463	18.73	+1.57	3.948	+2.68	4.002	+2.67
9	12,793	37,300	149.60	75.50	74.10	0.451	18.62	+0.98	3.864	+0.49	3.920	+0.56
10	15,304	50,800	161.90	77.75	84.15	0.440	18.05	−2.16	3.829	−0.42	3.874	−0.62
Average						0.451	18.44	±1.98	3.845	±0.94	3.898	±0.88

* The sum of measurements O and R (Du Bois) equals the distance from the symphysis pubis to the plantar surface of the foot.

average value 0.50 in the Du Bois series to the maximum 0.63. It is interesting to observe that the value of the exponent of the weight is quite different here from that in the formula derived from the Du Bois data. Whether or not this is to be related to the fact that the length of the body trunk relative to the total body length or height is greater in the Oriental than in the Occidental, is interesting to speculate upon. In this respect the "pattern" shape of the Japanese apparently tends toward the achondroplastic type.

There are a few other data available by which the principle of the new

expression—type 1, table 2—may be tested. MacLeod, Crofts and Benedict (1925) have published a study of the basal metabolism of nine Oriental women, and give body weight, body surface estimated by the height-weight formula of Du Bois, and sitting height. Using these data, the comparison of the new expression is with the Du Bois height-weight formula. As shown in table 7, the agreement is not unsatisfactory. Subject 6 differs by 8.35 per cent; none of the others shows a deviation of over 4 per cent, while the average deviation is ±2.27 per cent of the mean.

Wörner (1923) has published data on surface area measurements made by practically the same method as that employed by Du Bois and Du Bois (1915). The height, as well as weight and surface area, is recorded.

TABLE 7

DATA FROM ORIENTAL WOMEN (MACLEOD, CROFTS AND BENEDICT, 1925)				CALCULATIONS		
Subject number	Body weight W	Surface Du Bois height-weight formula S	Sitting height L	$\frac{W^{0.70}}{N_{obs}}$	$S = K W^{0.70} \sqrt{N_{obs}}$ K	d from average K
	grams	*sq. cm.*	*cm.*	N_{obs}		*per cent*
Chinese						
1	49,300	14,800	84.9	0.432	6.38	+0.47
2	54,500	15,700	88.2	0.430	6.28	−1.10
3	56,200	14,800	84.5	0.453	6.11	−3.78
4	44,500	13,900	86.6	0.409	6.10	−3.94
5	44,500	13,900	83.1	0.426	6.35	0
6	45,600	14,300	78.2	0.457	6.88	+8.35
7	49,600	15,500	88.2	0.417	6.41	+0.95
Japanese						
8	38,400	13,200	82.7	0.408	6.40	+0.79
9	42,200	13,500	83.3	0.418	6.28	−1.10
Average				0.428	6.35	±2.27

Unfortunately sitting heights, or stem lengths, or other measurements by which the latter could be determined accurately, are not given. We found it possible, however, by plotting Du Bois' data on height against stem length, to arrive at a simple expression by which stem length can be calculated from the height, namely:

(4) $$\text{Stem length}_{cm.} = 0.4\ \text{Height} + 10.5$$

Using this expression, the average deviation of calculated stem lengths from the measured was ±2.82 per cent of the measured value.

Table 8 presents Wörner's data and our calculations based thereon. It will be noticed that in only two cases are the deviations really serious,

and that only five out of the total of nineteen cases show a deviation greater than 5 per cent. The average deviation is ±3.54 per cent, practically identical with that given by the Du Bois formula. When one considers that stem lengths were calculated and not really measured, the agreement may be regarded as quite satisfactory. It appears significant to us that the average value of the constant here, namely, 5.367, should be so close to that yielded by the Du Bois data, of 5.384 (table 5).

TABLE 8

DATA OF WÖRNER (1923)					CALCULATIONS					
							$S = K_1 W^{0.71} \cdot \frac{0.63}{N_{obs.}}$		$S = K_2 W^{0.425} \cdot Ht^{0.725}$	
Subject	Age	Body weight W	Surface area S	Height Ht	Length $(L = 0.4Ht - 10.5)$ L	$\frac{W^{\frac{1}{3}}}{L}$ $N_{obs.}$	K_1	d from average K_1	K_2	d from average K_2
	years	*grams*	*sq. cm.*	*cm.*	*cm.*			*per cent*		*per cent*
1	5	21,800	8,439	110.5	54.7	0.511	5.690	+6.02	3.991	+6.23
2	9.5	28,400	9,947	130.5	62.7	0.487	5.297	−1.30	3.728	−0.77
3	14	31,500	11,015	140.5	66.7	0.473	5.276	−1.70	3.743	−0.37
4	14	37,100	12,584	145.7	68.8	0.485	5.521	+2.87	3.893	+3.62
5	17	42,300	13,333	154.5	72.3	0.482	5.297	−1.30	3.732	−0.67
6		50,000	14,440	152.3	71.4	0.516	5.454	+1.62	3.803	+1.23
7		64,300	15,157	153.0	71.7	0.559	5.187	−3.35	3.574	−4.87
8	25	58,700	15,863	155.5	72.7	0.535	5.545	+3.32	3.845	+2.34
8a	25	66,100	16,530	155.5	72.7	0.556	5.518	+2.81	3.808	+1.36
9	47	54,000	15,174	155.5	72.7	0.520	5.466	+1.84	3.898	+3.75
10		62,200	15,041	158	73.7	0.537	5.062	−5.68	3.514	−6.47
11	50	66,100	16,498	163.5	75.9	0.533	5.280	−1.62	3.665	−2.45
12	26	60,000	14,985	164	76.1	0.514	4.843	−9.76	3.462	−7.85
13	20	62,500	18,406	166	76.9	0.516	5.931	+10.5	4.141	+10.2
14		56,900	15,830	168.2	77.8	0.494	5.221	−2.72	3.672	−2.26
15		69,000	16,420	169	78.1	0.525	5.020	−6.46	3.496	−6.95
15a		92,600	19,353	169	78.1	0.579	5.419	+0.97	3.637	−3.19
16		66,500	18,179	177	81.3	0.498	5.413	+0.86	3.803	+1.23
17		64,500	18,702	182	83.3	0.482	5.504	+2.55	3.883	+3.36
Average						0.515	5.367	±3.54	3.757	±3.64

Human infants. Tests of the type 1 formula on babies can only be made indirectly, because in none of the papers available are stem lengths or comparable measurements as well as surface and body weight given. However, it has been possible to show through data obtained by Spencer (1924) that in babies of both sexes ranging from 6.63 to 11.27 kilos body weight,

(5) $$\text{Stem length}_{cm.} = 0.65\ \text{Height}_{cm.}$$

When this expression was used to calculate stem lengths of the babies measured by Spencer, the average error was ±1.80 per cent of the measured values. By using this formula with the data of Lissauer (1903) and Pfaundler (1916), whose infants were smaller than those of Spencer from whom the expression was derived, it was possible to compare the new type expression for estimating surface with those of Du Bois and Meeh-Rubner. With Lissauer's data the new expression proves slightly superior to the other two, but none of the formulae is very satisfactory. With Pfaundler's data, the Du Bois formula is the best and our expression quite unsatis-

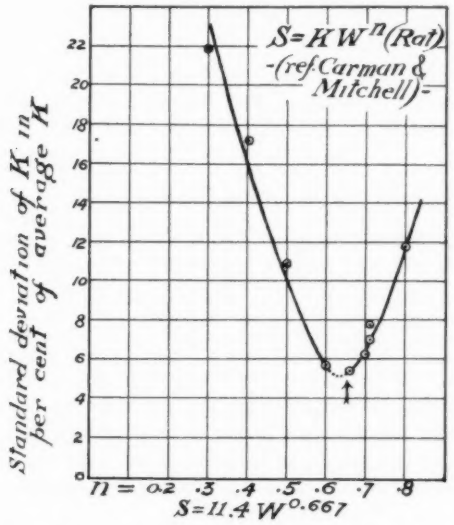

Fig. 5. This graph shows quite clearly that the best value for the exponent in a formula of the Meeh-Rubner type for the rat, using the data of Carman and Mitchell, is approximately 0.66.

factory, but again none of the expressions may be considered very good. In the light of these results it was not regarded profitable to consider additional data for babies secured by Meeh (1879) and others that might be mentioned.

Rats. The success which has attended the use of the rat for studies in nutrition has focussed considerable attention on this animal as a subject for metabolism experiments. Carman and Mitchell (1926) recently published skin measurements of sixty-two rats varying considerably in size, and on the basis of these data have fixed anew the value of the Meeh-Rubner constant for this species as 11.4. From our viewpoint it is un-

fortunate that length measurements comparable to those we made on the dog, were not made on these animals. The lengths as given—from the withers to the root of the tail—were found to have no value in any expression for surface such as the type 1 formula in table 2, or the Du Bois type. We have been able to show with Carman and Mitchell's data that 0.667—two-thirds power—instead of Dreyer's 0.70 to 0.72, is the best exponent to use for the weight. Figure 5 illustrates this fact quite forcibly.

Cattle. Data obtained with cattle were found in the papers of Hogan and Skouby (1923), Seuffert, Giese and Meyer (1926) and Brody and Elting (1926). Quite different methods were employed in obtaining these sets of data. Hogan and Skouby's tables include figures based on their own direct measurements of skins obtained at slaughter as well as similar data from Trowbridge, Moulton and Haight (1915). Seuffert, Giese and Meyer photographed the spread-out skin together with a measuring rod, and calculated the area by measuring the photograph and allowing for the extent to which the objects had been reduced in making the picture. Judging from the values of the nutritive index—$\frac{W^{1/3}}{L}$—given by these two sets of data, the animals measured were in various states of nutrition, some quite obviously thin, others obese. One gains the impression on reading the two papers just cited, that the areas of the hoof were not included in the totals given.

In table 9 are shown the data from the paper by Hogan and Skouby together with calculations of the nutritive index values, and constants and percentage deviations obtained using the formulae of Brody and Elting (1926), Hogan and Skouby, and a new expression based on the principle involved in our new formula for the dog. The extent of the individual variations is apparent upon inspection of the columns showing percentage deviations.

In the case of the data of Seuffert, Giese and Meyer, the agreement of the observed and computed values using a Meeh-Rubner formula could not be improved by consideration of length as well as weight. As compared with an average deviation of ± 4.93 per cent with the Meeh-Rubner expression, the Hogan and Skouby formula gave ± 6.16 per cent with one case showing $+24.7$ per cent and another -11.2 per cent deviation. The best application of our principle that could be made, yielded ± 6.82 per cent with three cases showing $+11.1$, -17.5 and $+23.8$ per cent deviations respectively.

Brody and Elting (1926) have published measurements of the surface area of large groups of pure bred Jersey and Holstein dairy cattle. The data were obtained by using a specially devised instrument consisting essentially of a wheel that is rolled over the surface of the animal, the areas represented by successive revolutions being summated as in an

TABLE 9

DATA OF HOGAN AND SKOUBY				CALCULATIONS						
				$S_{\text{sq. cm.}} = KW^{.562}_{\text{gm.}} \times \frac{0.65}{\frac{W^{\frac{1}{3}}_{\text{gm.}}}{L_{\text{cm.}}}}$ Cowgill and Drabkin		$\frac{W^{\frac{1}{3}}_{\text{gm.}}}{L_{\text{cm.}}}$	$S_{\text{sq. m.}} = KW^{0.56}_{\text{kgm.}}$ Brody and Elting		$S_{\text{sq. cm.}} = KW^{.4}_{\text{kgm.}} \times L^{.6}_{\text{cm.}}$ Hogan and Skouby	
Animal	Weight	Length "Withers to pin bone"	Area	K	d in per cent average K	$N_{\text{obs.}}$	K	d in per cent average K	K	d in per cent average K
	kgm.	*cm.*	*sq. cm.*							
1	568	156	55,407	26.35	−2.37	0.531	0.159	+0.57	211.9	−2.62
2	550	143	50,583	26.52	−1.74	0.573	0.148	−6.58	206.4	−5.15
3	486	140	47,721	26.26	−2.70	0.562	0.149	−5.57	206.7	−5.01
4	468	138	46,521	26.20	−2.93	0.563	0.149	−5.94	206.9	−4.92
5	406	132	45,171	27.47	+1.78	0.561	0.156	−1.07	218.3	+0.32
6	55	61	12,466	25.92	−3.96	0.623	0.132	−16.4	213.0	−2.11
7	131	95	23,581	25.81	−4.37	0.535	0.154	−2.72	218.3	+0.32
8	580	132	49,990	28.02	+3.82	0.632	0.142	−10.4	209.5	−3.72
528	688	172	64,028	28.60	+5.96	0.554	0.165	+4.36	213.8	−1.75
572	408	126	45,176	28.75	+6.52	0.589	0.156	−1.33	224.1	+2.99
573	318	125	41,140	27.94	+3.52	0.546	0.163	+3.29	226.5	+4.09
571	387	136	47,138	28.13	+4.22	0.536	0.168	+6.01	228.2	+4.87
574	276	125	38,236	26.83	−0.59	0.521	0.164	+3.92	222.9	+2.44
575	302	130	40,236	26.59	−1.48	0.516	0.164	+3.98	221.0	+1.57
577	511	150	55,664	28.27	+4.74	0.533	0.169	+7.15	227.3	+4.46
578	420	136	48,957	28.68	+6.26	0.551	0.166	+5.19	229.3	+5.38
579	480	150	53,190	27.40	+1.52	0.522	0.168	+6.01	222.6	+5.40
585	432	145	49,046	26.77	−0.82	0.521	0.164	+3.73	218.5	+0.41
503*	271	124	36,143	25.67	−4.89	0.522	0.157	−0.76	213.2	−2.02
509	439	147	49,701	26.66	−1.22	0.517	0.165	+4.17	218.3	+0.32
197	482	152	52,810	26.82	−0.63	0.516	0.166	+5.00	219.0	+0.64
507	457	153	50,175	25.69	−4.82	0.503	0.163	+2.85	211.8	−2.67
502	506	152	51,038	25.64	−5.00	0.524	0.155	−1.83	207.5	−4.64
541	324	111	38,036	28.97	+7.34	0.619	0.149	−5.50	223.3	+2.62
527	842	162	66,343	27.83	+3.11	0.583	0.153	−3.41	211.8	−2.67
515	743	150	58,846	27.43	+1.63	0.604	0.145	−8.16	206.9	−4.92
48	809	155	62,038	27.45	+1.70	0.601	0.146	−7.65	206.7	−4.97
501	883	152	64,635	28.59	+5.92	0.631	0.145	−8.47	210.4	−3.31
592	213	129	34,345	24.78	−8.19	0.463	0.171	+7.91	217.8	+0.09
558	108	87	20,189	25.22	−6.56	0.547	0.147	−7.21	213.8	−1.75
538	181	105	29,211	26.86	−0.48	0.539	0.159	+0.57	223.7	+2.80
524	362	145	46,417	26.38	−2.25	0.491	0.171	+8.41	222.0	+2.02
500	457	153	54,148	27.66	+2.49	0.503	0.175	+10.9	228.5	+5.01
540	158	99	26,068	26.23	−2.82	0.546	0.153	−3.23	228.7	+5.11
595	265	125	36,555	25.89	−4.08	0.514	0.165	+4.05	216.5	−0.51
525	305	131	39,955	26.15	−3.11	0.514	0.162	+2.72	217.5	0
523	381	136	46,827	28.05	+3.92	0.567	0.168	+6.26	228.1	+4.83
Average.....				26.99	±3.50		0.158	±5.22	217.6	±2.93

* Data below the line taken from Trowbridge, Moulton and Haigh (1915).

adding machine. Inasmuch as these data were obtained with pure bred animals, whose nutritive states as shown by the values of the nutritive index, were very much the same, and a formula of the Meeh-Rubner type seemed satisfactory, namely,

$$S = 0.15W^{0.56} \tag{6}$$

it was not considered worth while to attempt application of the length-weight principle. The fact that a formula of the Meeh-Rubner type should prove so satisfactory with these data further emphasizes the point that the individuals to whom a Meeh-Rubner formula is to be applied must have very nearly the same shape, if the expression is to be valid.

Swine. Using the data of Hogan and Skouby (1923), it was found impossible to construct a formula of our type giving better agreements than the expression arrived at by these authors, namely,

$$S_{\text{sq.cm.}} = 175\ W^{0.4}_{\text{kgm.}} \cdot L^{0.6}_{\text{cm.}} \tag{7}$$

In the case of the Seuffert, Giese and Meyer (1926) data, however, the Hogan and Skouby expression gave an average deviation of ± 5.38 per cent as compared with ± 3.45 per cent yielded by a new formula, namely,

$$S_{\text{sq.cm.}} = 10.72\ W^{0.63}_{\text{gm.}} \cdot \frac{0.33}{\frac{W^{\frac{1}{3}}}{L_{\text{cm.}}}} \tag{8}$$

Using this expression, the maximum deviations were $+6.7$ and -7.71 per cent.

Horses. Seuffert and Hertel (1924–25) measured the areas of the skins of seven horses and one suckling foal and determined the values of the Meeh-Rubner constant. The agreement was excellent. Using the live weights, and omitting the case of the suckling, the average deviation calculates to be ± 2.5 per cent, the average value of the constant being 9.98. Attempts to improve the agreement by introducing a linear measurement such as "Bandmasse" which we assume to be the trunk length, were unsuccessful. Our formula, that proved satisfactory for cattle (see table 9), gave an average deviation of ± 4.23 per cent, with maximum deviations of $+9.38$ and -7.71 per cent.

Chickens. Mitchell, Card and Hamilton (1926) have published measurements of the area of many chicken skins. Inasmuch as the error in the method was evaluated and found to be quite large, it did not seem profitable to use these data in making calculations similar to those just described for other species.

Discussion. A basis for understanding the rôle of height in the Du Bois formula is afforded by the fact that variations in surface area associated

with changes in the nutritive state can be so accurately measured by our nutritive state correction factor $\frac{N_m}{N_{obs.}}$, into which length enters and probably has the significance of a maximum diameter with which to correlate volume changes. So far as we are aware, no other explanation of the significance of height has been suggested heretofore.

The fact that the length weight formulae developed by consideration of the data for different species are not identical, does not seem to render invalid the principle upon which our new formula for the dog is based. The many groups of data were obtained following different methods. Some investigators have taken as the linear dimension for consideration trunk length, others stem length, and still others a total length such as height in man. It appears evident from the tables submitted that the total body surface area can be quite accurately estimated in many species by an expression of the types

$$S = KW^a \times L \tag{9}$$

of which our new formulae, illustrated by

$$S = K W^a \times \frac{N_m}{N_{obs.}} \tag{10}$$

are simply algebraic modifications.

In expressing the results of metabolism experiments made on other species than man or the dog, the investigator usually has animals differing in size as much if not more markedly than is the case with the species for which good expressions for estimating surface area are now available. As Harris and Benedict (1919) have pointed out, there is only a slight difference between the prediction values of surface and weight with human beings, and Murlin (1921) has shown why this should be so among subjects who do not differ to any great extent in size. It is quite evident that body weight can hardly be a satisfactory general unit of reference for expressing metabolism data when the experimental animals in the series are quite different in size. The value of Rubner's generalization regarding surface becomes evident here.[9] But suppose an investigator is working with a species for which no good formula for estimating surface area is available. Even Carman and Mitchell's formula for the rat is subject to a standard deviation of approximately ±5.4 per cent of the average; the greatest deviations from the mean were +14.3 and −13.7 per cent respectively. What, then, can be recommended as a unit of reference for the data?

[9] In making this statement we do not wish to be understood as endorsing wholeheartedly the original idea of Rubner as to *why* surface may be important in this connection.

Krogh (1916) has suggested that the "standard metabolism" be expressed in terms of the cube root of the weight and he is able to show a fair agreement of different species of animals when this unit is used.[10] This is an attempt to relate metabolism definitely to mass, a tri-dimensional entity, the unit of which would, of course, be the cube root.

Gulick (1915) expresses the heat production of rats in terms of what he calls a *metabolic index*, namely,

$$\frac{\text{Calories}}{\text{Weight}^{0.425} \times \text{Length}^{0.725}} \tag{11}$$

apparently assuming that the body surface of the rat is very nearly a linear function of $W^{0.425} \times L^{0.725}$, as it certainly is with human beings, this expression being simply the right hand part of the Du Bois height-weight formula without the equating constant. Our independent demonstration of the validity of such an expression, or what is practically its equivalent for determining the surface area of dogs, lends support to Gulick's idea.

In this connection it might be mentioned that Benedict (1924) has stressed the value of length and has pointed out the usefulness of von Pirquet's pelidisi concept as well. He particularly emphasized the importance of considering the *state of nutrition* when measuring basal metabolism. It will be noticed in our formula 1, table 2, that length is considered as a datum aiding in correlating variations in surface with variations in the *nutritive state*. With the metabolism shown in the previously discussed comparison to be so closely related to surface in two such widely different species as the dog and man, and length figuring so largely in correcting surface variations with variations in the nutritive condition, it does not appear surprising that the basal metabolism should be found to be so closely related to length. In our opinion, considering the present state of knowledge, it is more satisfactory to regard surface as the important factor to which to relate heat production, and to look upon length merely as an important datum of the same significance as a nutritional correction in height-weight formulae for determining surface. What the underlying significance of surface as related to metabolism may be, still is and doubtless will continue for some time to be without satisfactory explanation. Its prime usefulness as a practical standard, however, is undisputable.

In considering a suitable unit of reference in expressing the metabolism of animals for which no surface area formula other than one of the Meeh-Rubner type is available, the authors suggest 1, that the surface be assumed to be given by either of the following expressions:

$$S_{\text{sq.cm.}} = K\,\text{Weight}^{0.7}_{\text{gm.}} \times \frac{N_m}{N_{obs.}} \tag{12}$$

[10] See table XXXIX, page 141.

(the value for N_m being obtained by measuring a sufficient number of obese individuals of the species)

$$S_{\text{sq.cm.}} = K\ \text{Weight}_{\text{gm.}}^{0.425} \times \text{Length}_{\text{cm.}}^{0.725}; \tag{13}$$

2, that the metabolism be assumed to be more nearly proportional to surface area than to any other biotic measurement hitherto suggested; and 3, that for purposes of comparison of the data from individuals of the same species, the metabolism be expressed either as

$$\frac{\text{Calories}}{W^{0.7} \times \dfrac{N_m}{N_{obs.}}} \qquad \text{or as} \tag{14}$$

$$\frac{\text{Calories}}{W^{0.425} \times L^{0.725}} \tag{15}$$

The only effect that evaluation of constant K in the surface area formulae could have would be to change the *absolute* value of the metabolic index, and to enable one to express the metabolism in terms of true surface area. How far the above suggestions may with propriety be followed in comparing individuals differing widely in age, etc., is a question which probably can find no satisfactory general answer but will have to be left to the trained judgment of the investigator. The detailed studies of Benedict, Du Bois and their associates, and numerous other investigators of the variations of metabolism with age, sex, etc., in the human species, will furnish clues useful in interpreting the animal data, and need not seriously invalidate the use of these metabolic indices.

SUMMARY—CONCLUSION

The body surface areas of seven dogs ranging from 3.39 to 32.64 kilograms body weight and differing in nutritive states were measured by the paper mold-photographic method. Several formulae useful in estimating surface area were constructed on the basis of the data obtained. A formula new in its theoretical basis and consisting essentially of a Meeh-Rubner-Dreyer expression multiplied by a correction for the nutritive state of the subject, whether thin or obese, was devised. An expression for dogs similar to the Du Bois height-weight formula for humans was also invented.

The principle of the new type formula was tested for man by using the measurements of surface area of humans made by Du Bois and his associates, Wörner and Takahira, and new formulae for human beings based on this principle were developed, which proved almost as accurate as the Du Bois height-weight formula.

By means of the new surface area formula for dogs, it has been possible to make a new comparison of the basal metabolism of the dog and man. Rubner's generalization concerning the importance of surface area in relation to metabolism was demonstrated in striking fashion. Female dogs and women were shown to produce almost exactly the same number of calories per square meter of body surface in unit time.

The principle of the new type formula was also tested on data for cattle, swine and horses with satisfactory results. Similar tests using data for babies, rats and chickens were unsatisfactory.

It is suggested that in expressing the metabolism of animals for which no very satisfactory surface area formula has as yet been devised: 1, the metabolism be regarded as being closely proportional to body surface area; 2, this area be considered as a very nearly if not exact linear function of weight and length as expressed in the formula presented in this paper as well as by the Du Bois height-weight expression; and 3, the practice of Gulick be followed when making metabolism studies on animals for which no satisfactory surface area formulae are available, and metabolism indices be used based on the principles contained in these formulae for estimating surface area.

We desire to express our thanks to Prof. Graham Lusk for his courtesy in allowing us to publish new data recently obtained in his laboratory, and for his interest and encouragement. Thanks are also due Dr. H. J. Deuel, Jr., who measured the lengths of dogs XIX and XXVII for us.

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OBSERVATIONS ON THE CHEMICAL ACTIVITY OF THE SPLEEN

I. The Relation of the Spleen to Methemoglobin in the Blood

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A comprehensive review of the literature on the function of the spleen, such as that recently made by Krumbhaar (1926), indicates that although many facts have been acquired there are still large gaps in our knowledge, where the evidence is inconclusive or altogether lacking. It has been postulated that the spleen acts as a blood reservoir, that it may, under certain conditions, play a part in blood formation, and that it aids in blood destruction, probably both by rendering the cells more fragile and by destroying them through a phagocytic action of its reticulo-endothelial cells. Other functions have been ascribed to it, but those enumerated are the best known and the most widely accepted. The literature, however, fails to reveal any quantitative studies of the spleen in relation to intact incapacitated cells except the work of Barcroft and his associates (1924, 1925) upon the spleen following carbon monoxide poisoning. These workers arrived at the conclusion that one of the principal functions of the spleen is that of a reservoir which is capable of throwing fresh red cells into the circulation to replenish the supply of hemoglobin rendered non-functional by the gas. These observations are obviously concerned with the contractile or reservoir activity of the spleen and in no way indicate any possible chemical function the spleen might have in preventing the injury of the cells or in removing those already injured from the circulation.

If the hemoglobin of the red cells is so treated as to change the chemical nature of the blood pigment the question arises as to whether the spleen will have any effect upon the removal of the cells so injured, and if so, is it possible to determine the dynamic picture of its action as an organ of blood destruction. It is further interesting to determine the possible effect of the spleen upon cells that have been so injured. According to the evidence presented by Rous (1923) and Rich (1925), completely destroyed cells are removed from the circulation, but evidence is lacking in the case of

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those cells so treated as to change the hemoglobin without destruction of the morphological characteristics. While we may expect that the cells are removed from the circulation it is also desirable to determine if any pigment is reconverted to the oxygen bearing form.

There are certain drugs, principally the nitrites, that are known to convert hemoglobin to methemoglobin in the blood. As far as we have been able to determine this activity is not accompanied by an intravascular destruction of the blood. The cells must, therefore, be destroyed in some other part of the body. It is the object of this paper to determine if possible the rôle of the spleen in the removal of these cells. This has been done by comparing the curves of the changes in hemoglobin content of the blood with those of the total blood pigment in the presence and absence of the spleen in animals that have been treated with a methemoglobin-forming drug. Nitrobenzol was used to produce methemoglobin in the experiments cited below. This drug was chosen for the purpose because the effect seems to be purely respiratory and to have a minimum of the circulatory changes common to the nitrite group. While central nervous system changes are observed in cases of nitrobenzol poisoning these may be concerned with asphyxia, and not with destruction of brain tissue since in our experiments recovery takes place with a return to normal oxygen capacity of the blood. While Dittrich (1892) found nitrobenzol produced methemoglobin in the blood of dogs, Van Slyke and Vollmund (1925) were not able to repeat this reaction in rabbits. Our own spectroscopic observations on the pigment changes in the blood of the dog agree with those of Dittrich.

TECHNIQUE. In order to determine changes occurring in the blood of dogs after the administration of nitrobenzol, the following methods were used. The oxygen capacity of the blood was determined by the constant volume method of Van Slyke and Neill (1924). The total blood pigment was measured by the cyanhemoglobin method devised by Stadie (1920). The difference between the total pigment and hemoglobin, as determined from the oxygen capacity, we have called methemoglobin in the experimental results. The red blood cells were counted at each reading. Following control readings from which a standard was made for the total blood pigment determination, the dogs were given nitrobenzol in oil[2] by stomach tube and observations made as frequently as possible. Preliminary experiments on the normal animal showed that the dose used (0.1 cc. per kilo) did not cause such severe central nervous symptoms as found by Dittrich. Two dogs developed partial paralysis with occasional convulsions within 24 hours after the administration of the drug, and in both the attack wore off in 2 days. Six dogs were splenectomized, three of which had received nitrobenzol some 10 days previously. Of the three who had

[2] Nitrobenzol was prepared by nitrating benzol, and purified by distillation. The product as used had a boiling point of 205°C.

already had the drug, one died in convulsions 2 days after the operation. The other two showed no ill effects. Five days after splenectomy a control sample of blood was drawn for cell count, oxygen capacity determination and total pigment. Nitrobenzol was then given and blood determinations made at varying intervals as before.

TABLE 1

Reaction of dog before and after splenectomy

Male. Weight 15.00 kgm.

DATE		TIME*	TOTAL PIGMENT	Hb	Mhb	R.B.C. / 10,000	REMARKS
3/16	10:00		16.70	16.70	0.0	608	Control sample
	11:10	0					1.5 cc. nitrobenzol in 10 cc. oil
	12:06	0.85	16.83	15.17	1.66	586	
	2:15	3.00	16.65	14.34	2.31	583	
	3:30	4.5	16.08	13.50	2.58	595	Odor of nitrobenzol in blood
3/17	9:55	22.75	16.65	11.62	5.03	500	Animal cyanotic
	2:30	27.25	15.53	12.48	3.05	540	
3/18	1:30	46.15	13.61	13.47	0.14	487	Paralysis of hind legs
3/19	1:45						Spastic paralysis. Convulsions when touched
3/23							Paralysis gone
4/2							Splenectomy, uneventful recovery
4/9	8:15		15.85	15.85	0	436	Weight 12.5 kgm. control sample
	9:00	0					1.25 cc. nitrobenzol in oil
	10:00	1	15.28	14.12	1.16	398	
	12:00	3	16.52	11.05	5.47	401	
	2:00	5	16.52	10.92	5.60	398	Very cyanotic
	4:00	7	16.52	9.54	6.98	396	Very cyanotic
	6:00	9	16.18	8.65	7.53	364	Very cyanotic
	9:00	12	17.42	8.42	9.00	440	Very cyanotic, paralysis
4/10	8:00	23	16.28	9.00	7.28	404	Very cyanotic, paralysis
	10:00	25	15.39	9.21	6.18	364	Very cyanotic, paralysis
	12:00	27	15.70	10.00	5.70	414	Paralysis
4/11	2:30	53.5	16.62	11.85	4.77	526	Paralysis
	4:30	55.5	17.04	13.05	3.99	492	Paralysis

* Time refers to the duration of the nitrobenzol reaction.

EXPERIMENTAL RESULTS. A picture of the reaction of a single dog to a dose of nitrobenzol, before and after splenectomy, is shown in table 1. The figures given in the column marked "Time" refer to the hours' duration of the nitrobenzol action as we have observed it. As we have mentioned above, methemoglobin signifies the difference between the total blood pigment and the hemoglobin as determined from the oxygen capacity.

We have no evidence that all of the non-functional blood pigment was methemoglobin other than the qualitative spectroscopic readings.

The first portion of this table refers to the reaction of the normal animal. We find during the first $4\frac{1}{2}$ hours of the experiment the total blood pigment decreases gradually. The next day at 22.75 hours there is a slight increase over the preceding day which is not shown in the reading made at 27.25 hours. At 46 hours the total blood pigment shows nearly 20 per cent decrease from the control reading. It is interesting to follow, in comparison with this, the changes in the number of red cells. During the first period of the experiment (0–4.5 hours) there is an insignificant change in the number of red cells. At the same time the total blood pigment has fallen 4.5 per cent. The next day we find an increase in total blood pigment but a drop in the number of red cells. In general, however, the tendency is toward a decrease in the cell count corresponding to the decrease in total pigment.

Hemoglobin disappears for the first 22.75 hours at which time nearly one-third has been removed. During this period the total pigment of the blood has shown very minor variations. After the fourth reading we find a tendency for the hemoglobin to return to normal, although in the time remaining for this experiment, nearly 24 hours, less than 2 grams has been reconverted or replaced. At the final reading of this series we find that, while the above mentioned increase does take place, the total pigment has dropped to a value almost coinciding with the hemoglobin.

By reason of the method of measurement, the methemoglobin may be expected to vary as the difference between the total blood pigment and hemoglobin. Up to 22.75 hours we have what might be termed the period of formation. Since the total pigment is constant and the hemoglobin content decreasing it is obvious that very little methemoglobin is being removed. It is, indeed, probable that this period lasts far beyond this time as will be shown in a consideration of the curves representing the average of all the experiments. Following this phase is the period of recovery. The decrease in the methemoglobin in this period may be due to two factors. There is certainly a removal of this substance, since total pigment decreases at the same time. There is also the possibility that some of the methemoglobin is reconverted to hemoglobin since we find an increase in the oxygen bearing pigment. This may, however, be due to the formation of new cells.

It is interesting to note that this is one of the animals in which central nervous symptoms appeared. As mentioned in the protocol the animal developed a spastic paralysis which first appeared in the hind legs. That this was not due to destruction of brain tissue is shown by the fact that 4 days later the animal had entirely recovered.

Seventeen days after the dose of nitrobenzol the spleen was removed.

Recovery was uneventful and 1 week following the operation a second dose of nitrobenzol was given. It is interesting to note that neither the red cell

TABLE 2

Effect of first dose of nitrobenzol on splenectomized dog

Male. Weight, 12.00 kgm.

DATE		TIME*	TOTAL PIGMENT	Hb	Mhb	R.B.C. / 10,000	REMARKS
3/30							Splenectomy. Uneventful recovery
4/5	8:45		15.80	15.80	0	598	
	9:40	0					1.2 cc. nitrobenzol in oil
	10:50	1^{10}	17.18	15.03	2.15	683	
	1:35	3^{55}	17.18	13.88	3.30	494	
	3:30	5^{50}	17.75	12.97	4.78	487	
	5:35	7^{55}	17.96	12.25	5.71	541	
4/6	9:40	24	16.46	9.46	7.00	466	
	11:40	26	17.19	13.08	4.11	500	
	1:40	28	17.36	11.77	5.59	432	
	3:40	30	18.37	13.34	5.03	416	
	5:40	32	18.82	12.85	5.97	423	

* Time refers to the hours duration of the nitrobenzol reaction.

TABLE 3

Control dog

Male, weight, 9.8 kgm.

DATE		TIME*	TOTAL BLOOD PIGMENT	Hb	Mhb	R.B.C. / 10,000	REMARKS
4/19	9:00		15.96	15.96	0	488	Control
	10:00	0					1 cc. nitrobenzol in 20 cc. oil
	3:15	5.15	14.66	10.96	3.70	458	
4/20	10:00	24	16.12	11.06	5.06	470	
	3:00	29	14.12	11.24	2.88	424	
4/23							Laparotomy control operation
4/27			11.02	11.02	0	470	Control reading
4/28	8:30	0					1 cc. nitrobenzol in oil
	2:30	6	9.68	6.87	2.71	318	
4/29	9:00	24.5	11.49	8.84	2.65	386	

* Time refers to the hours duration of the nitrobenzol reaction.

count nor the hemoglobin content of the animal had returned to the value found in the first control reading.

One hour after the administration of the drug we find the hemoglobin content decreases nearly 7 per cent. This decrease continues for the first

period of the experiment (12 hours) at the end of which we find over 40 per cent of the hemoglobin is converted to methemoglobin. For the remainder of the experiment there is an increase in hemogobin content which returns to 82 per cent of the value found in the control reading.

It is noteworthy that the total blood pigment shows only minor variations during the period of experimentation, and that these variations are generally in the same direction as those occurring in the cell count. The result of the relatively constant total pigment and the variable hemoglobin is, during the first 12 hours, a considerable production of methemoglobin. The decrease, shown later in the experiment, is neither as great nor as rapid as in the normal dog.

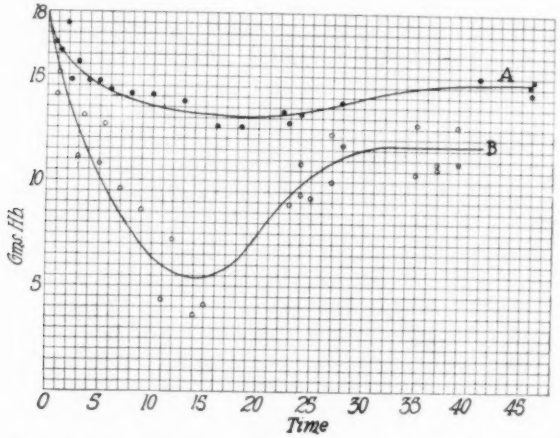

Fig. 1. Changes in the hemoglobin content of the blood of normal and splenectomized dogs following the administration of nitrobenzol. Curve *A* shows the reaction of the normal animal, *B* that of the splenectomized. Time in hours.

It may be suggested that the above results are due to the fact that the first dose of nitrobenzol influences the second. That this is not so and that a dog not previously treated will react in a similar manner is shown in table 2. While it is not exactly comparable, owing to the variation in time of sampling, we find that the characteristics of the data are those of the second part of the experiment given in table 1.

Further evidence that splenectomy produces an increased reaction is shown by the results found in the control dog, presented in table 3. The extremely constant results, shown in the curves, seemed to indicate that in such a check reaction a few readings, taken at the proper times, would show the nature of the blood changes. It will be observed from table 3 that the second dose of nitrobenzol gave changes comparable to the first.

On the same animal, in order to rule out effects of anesthesia and other disturbances due to surgical manipulation, a control operation (laparotomy) was performed. The second dose of nitrobenzol was given at the same time following the operation as in the splenectomized dogs. Neither the second dose nor the operative technique *per se* increased the amount of methemoglobin formed by the action of the nitrobenzol.

The changes in the hemoglobin content of the blood after the administration of nitrobenzol to normal dogs are shown in figure 1, *A*. The curve is determined by data obtained from 7 experiments. The points on the curve have been adjusted to a standard amount of hemoglobin (18 grams per cent) in order that the determinations made on various dogs may be

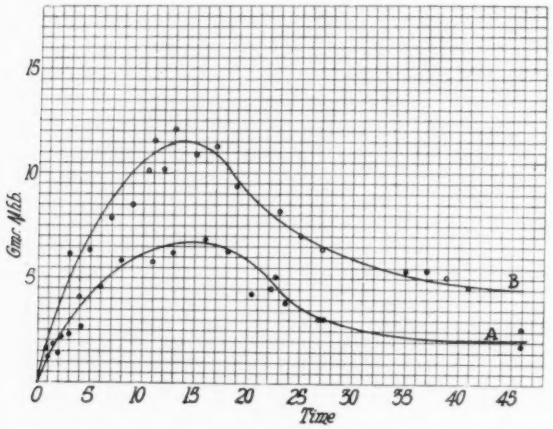

Fig. 2. The appearance of methemoglobin following nitrobenzol treatment. Curve *A* is the reaction of the normal dog, *B* that of the splenectomized. Time in hours.

comparable. As may be seen, there is a decrease in the oxygen carrying pigment of the blood which reaches its maximum at the end of 15 hours. It will be noted that the decrease is about 33 per cent of the total hemoglobin. After this the curve tends to approach the normal gradually, and at the end of 32 hours the hemoglobin content of the blood has returned to about 84 per cent of the normal amount. This concentration is maintained for several days. Although the points are not given in the curve, certain experiments were carried out over a period of 130 hours. In no case had the hemoglobin content returned to normal.

Curve *B* of figure 1 shows the results of similar experiments on splenectomized animals. It is similar in contour to the curve found in the case of the normal animals, but the decrease in hemoglobin content is decidedly

greater, reaching a maximum of 72 per cent in 15 hours. The maximum recovery is apparently in about the same time as in the case of the normal dog. It will be observed, however, that the amount of hemoglobin at this point is less than in the normal curve.

Figure 2 presents the curves of methemoglobin content of the blood of dogs following the administration of nitrobenzol. Curve *A* represents the formation of methemoglobin in normal dogs following the standard dose of the drug. It is a rounded curve reaching its peak at about 14 to 15 hours, then dropping to about 2 grams at the conclusion of the experiment. Curve *B* pictures the formation of methemoglobin in splenectomized animals after similar treatment. It can be seen that this curve is somewhat different in contour and rises to a higher peak. It reaches its maximum at about the same time as does the other but at a much higher level, and falls away gradually to about 5 grams of methemoglobin at the end of the experiment. In one instance observations were made 96 hours after the drug had been given, at which time a concentration of 5.75 grams per cent of methemoglobin was found. As has been said, two of the dogs received a dose of nitrobenzol 10 days to 2 weeks before splenectomy. Three others had had no previous dose. No difference could be made out in the reaction of the blood of either group to the administration of the drug 5 days after operation, but both dogs who had the second dose developed paralytic symptoms within 24 hours.

It is interesting to observe, in passing, that two dogs, one from the normal and one from the splenectomized group, failed to react as strongly to the drug as did the others, i.e., the formation of methemoglobin in each case was less than in the other members of the group. Nevertheless, in the case of the splenectomized animal the points fell well above the curve for normal dogs. Both dogs were exceptionally fat. One other splenectomized animal, which was pregnant, reached the peak of methemoglobin formation considerably earlier than the other dogs of the same group. She was obviously sick and had much pus in her urine 2 days after she had been given the drug. At autopsy no evidence of operative infection was found.

Discussion. The appearance of methemoglobin in the blood of the experimental animals is obviously the result of two processes. There is that process concerned with the production of methemoglobin which, we may assume, is the reaction between the hemoglobin of the blood and nitrobenzol plus some other factor. We must assume that there is some other body factor concerned in the production of methemoglobin inasmuch as the reaction occurs but rarely in shed blood according to the experiments of Dittrich (1892), and of Van Slyke and Vollmund (1925). The second process is that which is involved in the removal of methemoglobin. This removal may occur in two ways; one which involves the destruction of the

methemoglobin-bearing cell, and the other the reconversion of the transformed pigment into hemoglobin. In other words, it is possible to postulate a phagocytic action and a purely chemical reaction, the latter being most probably the action of some active reducing agent. How these reactions are brought about and their exact nature cannot concern us in this paper. It must suffice for the present discussion that we consider the formation and removal reactions without regard for their exact nature.

Obviously the reaction in the splenectomized animal is similar in nature to that occurring in the normal, the chief difference being that of magnitude. It is our concern as to what phase of the total reaction this difference in magnitude is due. There are three possibilities that suggest themselves as explanations of this phenomenon. 1. The removal of methemoglobin may be uninfluenced by the spleen, the increase in amount of this substance being an expression of an increased rate of formation. 2. The formation of methemoglobin may be constant whether or not the spleen is present, the observed difference being purely a function of the removal of the defunct blood pigment by the splenic and other tissues. Finally 3, both phases of the total reaction may be influenced by the removal of the spleen, the process of formation being accelerated and the removal inhibited.

That the removal of the spleen results only in an accelerated formation of methemoglobin and has no effect on its disappearance does not seem probable because of the following experimental evidence. In table 1 the blood count of the normal animal shows a decrease of 20 per cent from the normal at the end of the experiment and the total pigment has fallen 18 per cent. On the basis of this we may assume that red cells are being removed from the circulation. At the same time we find that methemoglobin has disappeared in about the same proportion. Over a corresponding period the splenectomized animal shows no such decrease, the blood pigment and red cell count remaining at almost a constant level. From these data which are illustrative of all the experiments, with the exception of those noted in the text, we suggest that the spleen does have a rôle in the removal of intact incapacitated red cells and the first possibility, postulated above, is not plausible.

The evidence that has been presented against the view that the spleen has no effect upon the removal of methemoglobin applies equally to the argument that the removal of methemoglobin by the spleen is the determining factor in the difference observed between the two curves. Certainly this must be considered as one of the possibilities.

The third view, that which supposes that the spleen is concerned with the formation of methemoglobin and its subsequent removal, is perhaps the most tenable. If the spleen were concerned with the disappearance of methemoglobin alone it is rather difficult to explain the fact that in the

splenectomized animal the down slope of the curve, which would indicate a preponderance of the removal reaction, shows us that the amount of methemoglobin removed per unit time is only slightly less than the normal. To be sure, the removal reaction lasts longer but this may be assigned to the increased amount of methemoglobin present. For this reason, if for no other, we cannot consider that the removal of methemoglobin is greatly accelerated by the presence of the spleen, but the evidence cited in table 1 shows that the spleen does play some part in the removal reaction. Since this is the case a portion of the observed increase must be assigned to the fact that the spleen is able to inhibit the formation of methemoglobin. We must, therefore, assume that there are other mechanisms that may remove methemoglobin from the blood. Suppose that these mechanisms are slower in starting to remove the non-functional blood pigment than the spleen, but once they have started are able to perform the task with considerable efficiency. In this case we may suppose the result would be a change such as we have found in the experimental data. If this were the case, however, it is rather difficult to conceive why the removal process is not far more efficient in the presence of the spleen than our experimental results seem to indicate.

There is a further point in support of the concept that both the formation and the removal of methemoglobin are influenced by the spleen. The maxima of the two curves, the normal and splenectomized, practically coincide in time. This would seem to indicate that the slope of the curve indicating the formation phase of methemoglobin must be increased in splenectomized animals.[3] We must, therefore, consider two possibilities in relation to the spleen and methemoglobin. Either the spleen is only concerned with the removal phase, for which view there is some support, or with both the formation and the removal, which concept seems necessary to explain our data.

The ways in which the spleen may act to inhibit the formation and hasten the removal of methemoglobin are open to conjecture. Several possibilities suggest themselves in the light of what is already known of the splenic functions. As a reservoir it may throw into the circulation a supply of uninjured cells to replace those in which the oxygen capacity is impaired. If the fresh supply of cells keeps pace with the number of injured cells removed from the circulation there should be little or no change in cell count and total pigment. There might be an initial rise in count if the reserve cells are thrown into the circulation faster than the injured cells are removed. Our results are so varied that no definite conclusions can be drawn except that there is no constant factor influencing this particular phase of the reaction. An anemia following the administration of nitro-

[3] For a discussion of curves of this type see Federlin (1902), Zeitschr. f. physik. Chemie., xli, 565.

benzol in the splenectomized dogs would suggest the absence of reserve cells. There is a tendency to such an anemia but it is neither sufficiently marked nor constant to warrant the assumption that the supply of fresh cells is much diminished. All that we feel justified in concluding is that a reservoir action may play a part in this reaction but only to a small extent.

To the spleen is also attributed the function of stimulating the formation of red cells. In our experiments we have seen no evidence of nucleated or reticulated red cells, following the first dose of nitrobenzol, though search has been made. As has been previously stated, there has not been sufficient difference in the reaction of the red cell count after nitrobenzol, before and after splenectomy, to justify any conclusions. Should such stimulation of new cell formation be going on it must be in a minor degree and overshadowed by some other action.

A third possibility of the action of the spleen in methemoglobin formation is that of its blood-destroying power. By phagocytic action, by fragmentation, or by rendering the cells more fragile, this organ may aid in the removal of the injured cells causing the disappearance of methemoglobin. That the absence of the spleen tends to diminish the rate of removal has been pointed out in the discussion of the curves and suggests that the blood-destroying power of the spleen plays an important part in the reaction in question.

If the spleen aids in the removal of methemoglobin by destroying the injured cells, how does this organ inhibit its formation? As neither its reservoir action nor its stimulation of red cell formation plays a sufficiently important part in this reaction, as has been pointed out, some other function must be suggested. Methemoglobin is the oxidation product of hemoglobin and is not found in the normal blood stream. That certain tissues of the body are capable of reducing methemoglobin to hemoglobin has been pointed out by Neill (1925). If the spleen is capable of active reduction it would tend to inhibit the formation of methemoglobin, and the removal of the spleen would remove this inhibition, and increase the rate of formation. Such reducing action would also tend to hasten the removal of methemoglobin.

Conclusions. Our experiments show that the spleen plays some part in preventing the formation and hastening the removal of a non-oxygen bearing blood pigment formed by the action of nitrobenzol. This pigment, in view of spectroscopic examinations, we have considered to be methemoglobin. We have suggested that the splenic functions of acting as a blood reservoir and stimulating red cell formation are of less importance in this particular reaction than its function of blood destruction. Finally, we have postulated another function, that of active reduction of methemoglobin to hemoglobin.

SUMMARY

1. Administration of small doses of nitrobenzol to dogs by stomach tube will produce methemoglobin.

2. Either the formation of this substance is hastened or its removal is retarded or both reactions are affected by splenectomy.

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SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD, ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

I. Chlorides

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A great deal of work has been done in studying the constituents, with their relative concentrations which are common to the urine and the blood. The literature, however, reveals little light on the comparison of the sweat with the urine, or the sweat with the blood, in this respect. What has been written as to these last named relations might be regarded as conjectures rather than facts supported by substantial evidence. As far as we have been able to ascertain, the literature is silent on a like comparison of the urine, the sweat and the blood, especially under well-controlled conditions. It seemed best to us that as many of these constituents as possible should be studied simultaneously. To that end each collaborator made his tests from the same sample of these fluids. Furthermore, necessarily all analyses were started as soon as samples were procured.

As the experiments progressed, our purpose was not only to study the relative amounts of the constituents of the above-named fluids but to study other questions that arose, viz.: What effects might sweating in itself have on the concentrations of these constituents in the blood and urine? What other manifestations in the blood might result from sweating? Would there be any change in the specific gravity? If so, could it be due to the greater concentration of the crystalloids or the colloids? Would it have any effect on the alkali reserve, gastric acidity and the metabolic rate? All of these have required more or less attention and will be reported in subsequent articles.

This work was started the first of June, 1925, and continued without interruption every day, including Sundays, up to August first of the same year, during which all of the available time was devoted to the collecting of data. At this time there were three investigators engaged with the problem. The work was so divided that one made the determination of the chlorides, another the total nitrogen and still another the urea and

ammonia nitrogen. Most of the time was devoted to the sweat and the urine.

There were forty experiments in the simultaneous study of the sweat and the urine. In six of these blood tests were also made. Besides these, there were more than a dozen control experiments where the urine was tested without sweating. One subject served thirty-one times. For the remainder, there were used three men serving one, three and five times respectively. Our principal subject (F. C.) was a janitor who did extremely hard work, while the others were students living more or less of a sedentary life. The subjects were used either early in the morning or late in the afternoon. No control was made of the diet.

During the summer of 1926 the same period of time was occupied, but the force of collaborators was increased to eleven for the purpose not only of extending the work of the previous summer but for the study of other constituents and manifestations resulting from sweating. This became especially suggestive as the blood was now included as a regular routine with the urine and the sweat. Furthermore, separate investigators took up the study of the sugar, amino acid and uric acid, after they had been discovered as constant in the sweat of normal individuals. Also regular assignments were made for the study of the specific gravity, red count and carbonates of the blood as well as the total and free acidity of the gastric content.

In these experiments we employed seventeen different subjects who, with one exception, served one to four times. This one was used twelve times and happened to be our principal subject of 1925.

These experiments were better controlled than those of the previous summer inasmuch as all were placed under the same condition as nearly as possible. Each was requested to appear early in the morning without partaking of food or water but was allowed to pass the night urine. An Ewald test meal was immediately given and then he was caused to swallow a modified Rehfus tube, which was retained for the entire time of the experimentation, as part of the procedure was for the study of the gastric acidity at regular stated intervals.

The method used for the production and collecting of the sweat was, with slight modifications, the same as reported by one of us (1). It is well to mention that most of the sweat was produced by moist heat in a cabinet, although dry heat was employed in one instance. In ten cases sweat was produced by work. Before entering the cabinet the subject gave a sample of urine and also a sample of blood which was drawn from the basilic vein. Both of these were used as controls. The skin was prepared as previously reported. The subject remained in the cabinet from a half to three-quarters of an hour, depending upon the conditions favorable for sweating. At the end of this period we were usually able to

collect 50 to 150 cc. of sweat. After retiring from the cabinet, another sample of urine was taken followed by two or more at hourly intervals. Also at this instance another sample of blood was drawn from the basilic vein of the opposite arm from which the control sample was taken. In both instances the blood was discharged into potassium oxalate and deproteinized according to the method of Folin and Wu (2).

DETERMINATION OF CHLORIDES. Our purpose in this article is to confine our attention briefly to the data obtained on the chlorides alone, reserving for later articles the charting of this against the other constituents.

The method (3) used for the determination of chlorides in the sweat and urine was by precipitation with standard silver nitrate and the titration of the excess of silver nitrate by a standard ammonium sulphocyanate, using ferric ammonium sulphate as an indicator. The blood filtrate chlorides were determined by the Whitehorn method (4) in which potassium sulphocyanate is used as a titer and powdered ferric ammonium sulphate as an indicator.

We found that the chlorides of the sweat varied from 4.3 to 8.3 mgm. per cc. The average was from 5.5 to 6.5 mgm. per cc. with the sweat produced in rather a moist atmosphere. We might have expected a little different concentration if we had employed dry heat, as is produced by electric light baths. From our observation we have not as yet been able to discover any definite law as to the concentration of the chlorides in its relation to the profuseness of the sweating, as reported by Kittsteiner (5) and Viale (6) who claim that the concentration increases with the profuseness. It is only in a rough manner that one can ascertain the total quantity of the sweat. Furthermore, it is a well-known fact that individuals differ in the profuseness of the sweating in the various parts of the body. Also one of us (7) has hitherto reported that there is a regional variation in the per cent of solids which presumably is due chiefly to the chlorides.

We have made over 400 determinations of the chlorides of the urine from twenty-one different subjects, all of whom were normal. Under our conditions of experimentation we have discovered a normal variation from 5 to 12 mgm. per cc. In about 60 per cent of the cases there was a fall in the chlorides in the second sample which was taken immediately after sweating, followed by a rise in many instances in the third sample. In some cases the fall was not noted until the third sample which might be due to the lag in the secretion.

At first we were rather inclined to believe that this fall in the concentration of urine chlorides was due to the sweating. It seems, on the other hand, that rest is the factor that causes the fall, for a person sitting in the cabinet is more or less relaxed. Furthermore, in our control experiments where the subject sat in the cabinet for the usual time without sweating there was the same fall in the second sample. We have also noted in the

TABLE 1

(1925)

Chlorides in urine and sweat in milligrams per 5 cubic centimeters

SUBJECT	URINE 1—NORMAL	URINE 2—IMMEDIATELY AFTER SWEATING	URINE 3—1 HOUR AFTER SWEATING	URINE 4—2 HOURS AFTER SWEATING	SWEAT
F. C.	50	50	56		26.6
	43	44	51		25.2
	38	44	54		26.7
	26	31	54		24.6
	34	49	58		26.1
	33	35	47	46	21.5
	39	29	42		26.0
	39	34	46	51	22.3
	51	36	46	51	22.4
	42	33	50	59	24.9
	50	43	49	54	24.7
	44	40	48	46	28.6
	41	43	39	43	29.8
	40	43	33		26.7
	48	44	45	44	25.9
	41	34	39	43	25.2
	41	41	42	45	29.5
	40	48	42	43	29.0
	51	49	49	48	32.5
	39	41	39	40	31.0
	30	29	34	42	26.3
	37	31	36		27.9
	40	38	41	44	29.3
	41	35	40	39	30.2
	32	39	40	47	31.9
	29	33	34	36	30.7
C. H.	44	45	45	48	27.8
	43	42	40	40	26.3
	44	43	39	40	26.5
	40	51	46	43	31.9
	42	43	39	44	32.5
C. O.	40	37	40	41	23.5
	45	30	37		23.5
	44	35	37	39	24.5
R. T.	39	36	34	33	28.5

case of F. C., who was a hard-working man, that there was a tendency to a rise in the urine chlorides when he was fatigued.

As to the blood filtrate, we discovered in our experiments that the chlorides ranged from 3.30 to 3.90 mgm. per cc. We have noted frequently a

TABLE 2

(1926)

Chlorides in urine and sweat in milligrams per 5 cubic centimeters

SUBJECT	URINE 1—NORMAL	URINE 2—IMMEDIATELY AFTER SWEATING	URINE 3—1 HOUR AFTER SWEATING	URINE 4—2 HOURS AFTER SWEATING	SWEAT
I. R.	52	58	57		
H. I.	37	37	39	45	25.7
F. C.	33	37	32	35	30.2
C. H.	53	53	53	50	
F. C.	39	48	50	54	31.4
W. G.	39	34	33	39	
S. S.	60	56	55	57	30.7
W. G.	29	35	37	38	24.7
J. A.	57	52	52	51	29.5
F. C.	42	41	39	40	32.0
L. M.	48	45	41	45	
F. C.	47		49	47	32.3
J. Mc.	41	44	42	37	
F. C.	35	35	35	41	33.0
J. A.	42	34	38	39	30.0
F. G.	54	32	40	37	41.5
F. C.	45	43	33	41	29.7
G. Mc.		37	28	35	29.8
G. B.	46	41	33	30	25.6
F. C.	44	46	39	49	30.0
J. A.	29	27	34	36	24.3
I. H.		41	35		26.2
W. K.	31	26	26	25	30.5
I. R.	57	57	57	45	27.8
C. H.	39		40		
F. C.	44	48	50		30.6
J. A.	42	42	48	46	28.0
M. A.					27.8
G. Mc.	45	45	45	44	
W. K.	47	45	44	51	31.4
I. R.	54	54	52	51	24.9
F. C.	38	32	29	27	30.1
W. K.	57	56	54	52	33.8
C. H.	37	35	42	35	
J. A.	50	47	47	40	29.3
F. C.					35.3
I. R.	56	55	55	54	27.3
F. C.	28	29	30	34	30.1
O.H.	58	56	51	44	25.1
J. A.	44	44	42	42	28.0
F. C.	33	33	35	44	29.0
C. H.	56	43	35		
C. H.	52	44			26.4

difference in the concentration between the control sample and the sample taken immediately after sweating. However, it is quite as apt to fluctuate one way as the other. The most regular increase in the specific gravity of the blood as a result of sweating then hardly seems due to the influence of the chlorides.

In table 1 are shown the data obtained from the sweat and urine analyses made during the summer of 1925 which, as previously stated, were mainly from one subject. In table 2 in a similar manner are given the data obtained during the summer of 1926 in which seventeen subjects were employed.

In figure 1 we give the chlorides of the sweat plotted against the chlorides of the urine. The lack of correlation is so obvious that no attempt was made to figure the per cent. In figure 2 the blood chlorides are plotted against the sweat chlorides. There is much more evidence of correlation

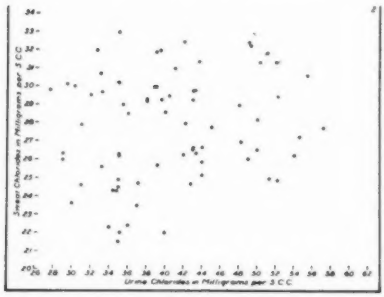

Fig. 1

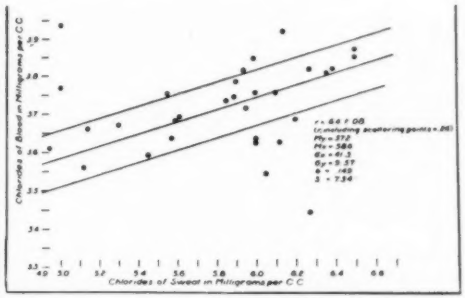

Fig. 2

here as is shown by the 64 per cent, with the omission of the two extremes above and one below the regression lines.

We wish to convey our thanks to Doctor Banks of the Department of Pathology and to Mr. Story of the Department of Public Health for valuable assistance, and to Doctors Abbott and Harris of the Chemical Department who kindly offered space in their laboratories during the summer of 1925, and also to F. Campos who acted sixty times as a subject.

SUMMARY

A simultaneous study of the constituents of the sweat, urine and blood has been made.

1. A general survey is presented of the experimental methods underlying this and other articles of the series which will appear subsequently.

2. Fluctuations in the chlorides of the sweat, urine and blood are noted.

3. There is no evidence of correlation between the sweat and urine chlorides.

4. There is considerably more evidence of a correlation between the blood and sweat chlorides.

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SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD, ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

II. Total Nitrogen of Sweat and Urine; Total Non-Protein Nitrogen of Blood

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For several years it has been known that nitrogenous bodies are excreted through the skin, but the experiments that have been made are rather few and far between.

Argutinsky (1), by two experiments in 1890, attempted to find the nitrogen content of the sweat. His method was to entrap the sweat in special clothing which had been washed and rewashed. The clothes were worn on a July day which was spent in walking. Toward evening the clothing was removed and soaked over night in N/10 oxalic acid and the nitrogen then determined in the acid. Urine also was kept for analysis throughout the day of the walk. He came to the conclusion that 4.7 per cent of the total excreted nitrogen was passed through the sweat glands.

Camerer (2) conducted four experiments with the production and analysis of sweat. In two of them sweating was induced by the use of incandescent lamps, in one by the use of hot air and another by the use of a steam bath. The sweat was caught in a receptacle and afterwards was absorbed by cotton from which the fat had been previously removed. He found that of the total nitrogen 34 per cent was urea and 7.5 per cent was ammonia nitrogen.

Kittsteiner (3) and later Viale (4) reported that in prolonged sweating the concentration of total nitrogen decreases thereby differing from the chlorides. We often found that these constituents ran inversely but it was by no means uniform.

Barney (5) found that the average total nitrogen per 100 cc. of sweat was more than twice that of blood. Our results seem to confirm his findings.

Methods. The method of inducing the sweating and the time of collecting the sweat, urine and blood was the same as reported in article I of this series. The method of analysis of the total nitrogen of the sweat

TABLE 1

1925

Total nitrogen and urine and sweat in milligrams per cubic centimeter

SUBJECT	URINE 1, NORMAL	URINE 2, IMMEDIATELY AFTER SWEATING	URINE 3, 1 HOUR AFTER SWEATING	URINE 4, 2 HOURS AFTER SWEATING	SWEAT
F. C.	13.9	15.4	13.2		0.99
	15.8	16.0	13.5		0.64
	12.9	11.9	16.9	10.8	0.65
	17.2	10.0	9.9		0.81
	10.8	18.1	14.0		0.62
	16.0	18.5	12.8		0.75
	7.8	14.0	9.7		0.75
	8.3	10.5	8.2	6.6	0.62
	11.3	17.5	12.9		1.63
	11.2	12.1	11.5	9.5	1.17
	14.2	16.7			0.83
	8.3	11.6	10.3	9.9	0.53
	14.1	14.7	14.5		0.68
	6.7	8.5	8.6		1.38
	6.8	12.5	13.1	20.6	0.58
	11.1	13.4	17.3	17.6	1.05
	12.0	11.9	11.4	11.6	1.60
	12.3	7.7	7.3	9.4	1.10
	12.1	7.8	12.1		0.48
	19.2	15.0	9.7		0.45
	19.2	13.1	15.2		0.34
	10.9	14.2	9.2		0.34
	9.0	10.1	7.8		0.76
	19.2	30.9	21.3		1.61
	18.9	21.9	14.3		0.90
	19.3	18.8	16.8	11.7	0.75
	16.6	16.9	13.8	15.1	0.83
	9.2	13.4	16.8	11.2	0.65
	16.9	13.7	14.8	15.0	0.67
C. H.	11.0	12.4	15.9		0.70
	13.6	14.0	14.1	11.9	1.30
	13.0	15.4	14.9	13.8	0.70
	11.2	11.5	12.8	11.6	0.72
	10.3	11.9	15.2	13.3	0.72
C. O.	12.9	13.5	15.6		0.80
	10.1	5.1	8.7		0.77
	8.3	8.6	15.6	10.1	0.53
R. T.	7.1	10.7	15.4	15.4	1.70

TABLE 2

1926

Total nitrogen of urine and sweat in milligrams per cubic centimeter, total nitrogen of deproteinized blood in milligrams per 100 cubic centimeters

SUBJECT	URINE 1	URINE 2	URINE 3	URINE 4	SWEAT	BLOOD 1	BLOOD 2
H. I.	21.3	19.8	17.6		1.1	27.0	32.0
F. C.	14.6	19.4	19.4	15.7	0.86	18.3	21.0
C. H.	11.4	12.3	15.9	18.8	1.3		
F. C.	8.4	8.3	7.9	5.9	0.47		
W. G.	5.1	4.3	8.0		1.00	23.8	25.4
S, S.	9.0	10.4	10.6	12.0	0.49	28.0	28.0
W. G.	15.4	15.3	15.0		0.50	21.6	19.7
J. A.	8.9	9.9	9.3		0.65		
F. C.	15.4	15.9	15.6	15.4	0.70	21.5	31.0
L. M.	10.2	11.0	13.0	11.2	0.80	32.0	29.0
F. C.	12.1	13.6	11.3	8.0	0.70		
F. C.	18.0	20.0	19.8	16.0	1.00	24.8	25.6
J. A.	3.6	5.3	5.6	4.3	0.47	33.9	36.0
F. G.	10.0	14.0	9.7	11.8	0.60	24.4	27.0
F. C.	19.8	7.6	11.8	13.2	0.70	26.0	28.0
G. Mc.	6.7	4.3	7.2	10.9	0.74	33.0	32.1
G. B.	15.0	11.1	12.5	14.9	0.81	32.4	25.0
F. C.	13.1	14.1	14.8	10.3	0.58	31.8	26.7
J. A.	4.5	8.7	9.6	8.1	1.00	24.4	40.0
I. H.	16.0	18.0	12.0	16.5	0.87		
W. K.	6.2	5.9	5.1		0.60		
R. B.	22.0	20.0	13.0			42.4	50.6
C. H.	22.0	24.5	21.8			26.1	35.1
F. C.	12.1	14.2	14.0		0.60		
J. A.	6.3	7.1	5.8		0.57		
G. M.	9.6	10.4	9.3				
W. K.	7.6	7.9	9.6		0.93	30.0	25.0
F. C.	15.0	13.3	20.0		0.80		
W. K.	12.3	14.4	12.9		0.75	46.2	39.9
C. H.	16.1	18.0	17.8		1.10	46.2	48.6
J. A.	10.3	9.6	13.3		0.55	46.0	43.5
I. R.	9.8	12.2	12.9		1.10		
F. C.	12.3	14.0	13.8		0.49		
O. H.	10.1	9.5	9.8		0.70	32.1	36.0
J. A.	16.8	18.1	18.9		0.60		
F. C.	13.3	17.0	20.4		0.61		
W. K.	14.6	13.3	13.0		1.00	31.4	32.1
O. H.	11.1	10.8	10.9		0.51	31.8	37.0

and urine was that of Folin and Denis (6) and the method of deproteinizing the blood and the determination of the non-protein nitrogen of the same was that of Folin and Wu (7).

Total nitrogen of the sweat. In 39 determinations, made chiefly on the

samples of one subject during the summer of 1925, we obtained from a minimum of 0.34 to a maximum of 1.60 mgm. per cc. During the summer of 1926, in which there were 40 determinations from 17 subjects, we observed a minimum of 0.47 to a maximum of 1.30 mgm. per cc. The average from all these observations was 0.8 mgm. per cc.

Total nitrogen of urine. Under the conditions of these experiments there were close to 400 analyses made of the urine in which there was found a variation from 7.8 to 30.9 mgm. per cc. It might be well to note in this connection that subject J. A. was a vegetarian. In 75 per cent of the sweat experiments there was a rise in the total nitrogen of urine from the samples taken immediately after sweating. We are unable to say at present whether or not this rise is induced by sweating.

Total non-protein nitrogen of the blood. Under the conditions in which these experiments were performed, we have discovered in 60 determinations upon 20 different subjects that there was a minimum of 18.3 to a maximum of 54.6 mgm. of non-protein nitrogen per 100 cc. of blood filtrate. It will be noted, in comparing the control with the sample of blood taken after sweating, that there is a fair amount of fluctuation one way or the other. As was said about the chlorides, it hardly can be conceived that this fluctuation of the total nitrogen is an influencing factor in the specific gravity of the blood.

In table 1 the comparative total nitrogen of the urine and sweat for the summer of 1925 is given. These data are mainly from one subject. In table 2 the total nitrogen of urine and sweat and the total non-protein nitrogen of the blood are given. These are the data for the summer of 1926 in which 17 subjects were employed.

As in the preceding paper, correlation of these findings was sought for. When, however, the values were plotted, *a*, total nitrogen of sweat against total nitrogen of urine; *b*, total nitrogen of sweat against total non-protein nitrogen of blood, and *c*, chlorides of sweat against total nitrogen of sweat, no outstanding correlations developed. There is a slight correlation between total nitrogen of sweat and urine, and some correlation between total nitrogen of sweat and total non-protein nitrogen of blood. With the available data, however, such correlations can only be regarded as suggestive.

SUMMARY

1. The variations in the total nitrogen of the sweat and urine as well as the total non-protein nitrogen of the blood filtrate are noted.

2. Barney's contention, that the total nitrogen per 100 cc. of sweat is more than double that of the total non-protein nitrogen of the blood, is confirmed in most instances.

3. There is only a slight correlation noted in the total nitrogen of the sweat and urine.

4. Some correlation is noted between the total nitrogen of the sweat and the total non-protein nitrogen of the blood.

5. No correlation is noted between the chlorides and the total nitrogen of the sweat.

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DURATION OF LIFE AFTER SUPRARENALECTOMY IN CATS AND ATTEMPTS TO PROLONG IT BY INJECTIONS OF SOLUTIONS CONTAINING SODIUM SALTS, GLUCOSE AND GLYCEROL

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There have been numerous attempts to prolong the life of suprarenalectomized animals by the use of epinephrin, extracts of whole gland and of cortex, all of which have yielded doubtful or negative results.

During the past three years we have also tried extracts of the suprarenal gland prepared in various ways on suprarenalectomized cats. Our results, using the duration of life as the test, indicated that certain preparations prolonged life, while others did not, but that the degree of prolongation might as well have been due to the water and sodium chloride injected with the extracts as to any substances peculiar to the extracts.

In order to ascertain more definitely the cause of the prolongation of life we have studied the effects of the intraperitoneal injection of sodium chloride solutions, Ringer's solution, Ringer's solution with and without calcium and potassium chloride, sodium acetate, sodium glycerol-phosphate, glucose and glycerol. The chief object of this paper, therefore, is to report the results of our work on the influence of the various solutions above mentioned, including water, on the duration of life following double suprarenalectomy, although it will be necessary to refer briefly to some of the work with extracts.

Cats were used for the reason that we desired a readily obtainable and easily operable animal in which double suprarenalectomy is regularly fatal. In order to make the suprarenal insufficiency more acute and severe as well as less complicated, we remove both glands at one operation. The rabbit and the rat are entirely unsuited for such experiments for the reason that under our conditions about 40 per cent of rabbits and 60 per cent of rats survive indefinitely the removal of both suprarenals.

The duration of life after suprarenalectomy is influenced by so many factors, notably by poor surgery and poor after-care, as has been emphasized by Stewart (1), that the results of most other workers are of little value in this connection. It was necessary to determine how long suprarenalectomized cats would live under the conditions existing in this labo-

ratory in order to evaluate any possible effects of the substances administered. As a result of our experience we believe that death with rare exceptions occurring within 48 hours after double suprarenalectomy at one operation may be regarded as due to trauma, shock and bad after-care. It is therefore unnecessary to review the literature since, in most of the experiments reported, death occurred within 24 hours.

There are, however, a few reports which should be mentioned. In Elliott's (2) series of 21 cats in which both glands were removed at one operation, 19 survived less than 48 hours. The other two lived 9 and 10 days respectively. In another series of 25 cats in which Elliott removed the suprarenals in two stages, the duration of life was longer. Marshall and Davis' (3) cats survived double suprarenalectomy (done in two stages) on an average of about 3.5 days, while 5 of the 16 lived from 5 to 7 days. His animals were subjected to manipulations necessary for the experiments being made and their lives were probably shortened because of this. Hartman's (4) series of 17 cats in which attempts were made to prolong life by the injection of epinephrin and of extracts of the suprarenal cortex lived on an average of 50 hours, while 22 other suprarenalectomized cats in which he injected salt solution extracts of the cortex lived on an average of about 6 days.

OPERATIVE TECHNIQUE, FEEDING AND AFTER-CARE. All animals were operated upon 15 or more hours after the last feeding. Anesthesia was started with chloroform under a bell jar. When the animal showed the first sign of relaxation it was quickly removed and the administration of ether begun by the open method. An area roughly 7×12 cm. was shaved in the midline of the back with its center at the level of the upper poles of the kidneys. The shaved area was cleansed with soap and warm water, washed with 1–1000 bichloride solution, 95 per cent alcohol and then painted with a 2 per cent solution of mercurochrome.

A longitudinal skin incision about 8 cm. in length is made in the midline. The animal is rotated slightly to the left side, the skin retracted and an incision roughly parallel with the last rib about 4 cm. in length is made through the quadratus and oblique muscles. This incision exposes the retroperitoneal tissues above the right kidney without opening the peritoneum. The peritoneum is gently retracted ventrally with the finger and a self-retaining retractor inserted which exposes the right suprarenal gland. (A pad of one or two folded towels inserted under the upper part of the animal's abdomen brings the suprarenal nearer the surface.) The right lumbar vein is divided between hemostats and its distal end ligated. The proximal end is used to steady and retract the gland during its isolation by blunt dissection. The pedicle is ligated with stout silk. Then with a little traction one can readily cut the pedicle close to the gland. The muscle wound is closed at once with silk sutures. The animal is then

rotated slightly to the right side. The skin is retracted on the left and a left muscle incision is made similar to that on the right. The left lumbar vein is clamped and divided and its distal end ligated. The gland is isolated, ligated and removed and the muscle incision closed exactly as on the right. The median skin incision is then closed either with interrupted or continuous or with a combination of interrupted and continuous sutures, care being taken to anchor the skin to the muscle fascia near the middle of the incision. The wound is then covered with squeezed-out bichloride sponges and a protective bandage is applied.

With a skilled assistant and a good anesthetist the whole operative procedure from beginning the chloroform anesthesia to placing the animal in its cage usually requires between 25 and 30 minutes, the difference depending largely on the amount of fat surrounding the suprarenal glands, which factor largely determines the time required for isolation. The peritoneum on the ventral surface of each gland is necessarily torn in isolating them for ligation. Occasionally a small opening in the peritoneum may be made by extending the muscle incision too far forward, otherwise the operation is entirely extraperitoneal.

All the operations reported in this paper were done by the same operator, assistant and anesthetist.

After operation the animals are placed in cages, well bedded with clean straw and in warm rooms. Nothing but water is given during the first 24 hours. Then a cup of fresh milk and a dish containing finely chopped lean meat or liver and some canned salmon are given. This constitutes the daily diet.

Period of survival of cats after double suprarenalectomy (control experiments). Double suprarenalectomy was performed on 30 cats to serve as controls. The operations were scattered throughout the three year period and we believe the results give a fair cross section or average of the stock of cats used, of any seasonal influence and of any possible variation in operative procedure. Of the 30 cats set apart for controls, 12 have been excluded because of the following complications: 5 were in various stages of pregnancy; 2 died within 12 hours of possible shock and trauma; 1 that lived 5 days was found at autopsy to have had its left renal vein nearly completely ligated; another died of acute bronchopneumonia after 2.8 days and another living 2.8 days had congenital absence of the left kidney; another cat that survived suprarenalectomy 12.5 days was excluded because a large accessory suprarenal was found. A kitten that survived 22.3 days was also excluded, although no accessory was found, because the result was so out of line with the others of this series as well as with our experience with several hundred other suprarenalectomized cats.

The 18 "uncomplicated" control cats lived 2.0, 2.5, 2.8, 3.0, 3.5, 4.0,

4.2, 4.3, 5.0, 5.2, 5.2, 5.5, 5.8, 7.0, 7.0, 7.5, 8.8 and 12 days respectively. The shortest survival period was two days, the longest 12 days, and the mean and the average duration of life were 5.2 and 5.3 days respectively.

Since some observers have claimed that thyroidectomy prolonged the life of suprarenalectomized animals it may be of interest to report incidentally the results we obtained with 3 cats which had been thyroidectomized some months before in connection with other experiments. In removing the thyroids care was taken to save the IIId parathyroids with their main blood vessels. In three instances we were successful as verified at autopsy. These animals were kept on the same stock diet throughout except that for a few days following thyroidectomy calcium lactate was administered. These cats survived suprarenalectomy 5.0, 6.1 and 6.0 days respectively. They were sacrificed the day after they had stopped eating and would have

TABLE 1

CAT NUMBER	SEX	AGE*	EXTRACT INJECTED	PERIOD OF SURVIVAL
				days
92	F	2	Extract 30	9.0
91	M	4	Extract 33	8.8
94	F	3	Extract 34	8.5
96	M	4	Extract 34	17.0
105	M	2	Extract 36	10.5
106	M	3	Extract 36	16.5
116	F	1	Extract 38	11.9
118	F	3	Extract 38	14.0
113	F	2	Extract 38a	11.5
114	M	3	Extract 38a	11.8

* In this and all succeeding tables the approximate age has been indicated as follows: 1 = kitten, 2 = young adult, 3 = middle aged adult, 4 = old adult.

lived perhaps ½ day longer. There was, therefore, no significant prolongation of life, nor did we see any prolongation of life in a series of 15 thyroidectomized rabbits previously reported (5).

THE EFFECT OF EXTRACTS OF THE SUPRARENAL GLAND ON THE SURVIVAL PERIOD OF SUPRARENALECTOMIZED CATS. Many extracts have been prepared for a study of their effects on metabolism but relatively few have been studied from the standpoint of prolongation of life. The details of the methods of preparation need not be discussed here. In all cases the extracts were sterilized by passing through Berkefeld filters.

An alkaline extract of the suprarenal cortex freed of most of its proteins whose reaction had been adjusted to pH 4.5 to 5.0, was injected twice daily into a suprarenalectomized cat. It lived 2.5 days and as it seemed definitely to shorten life no further attempts were made.

A number of hydrochloric acid extracts of suprarenal cortex were made. The hydrochloric acid was neutralized with NaOH, which at the same time removed most of the proteins. These extracts were in some instances concentrated under diminished pressure to a small volume and treated with 8 or 10 volumes of 99.8 per cent alcohol. The precipitate was centrifuged off and washed several times with alcohol and ether and when used was dissolved in 0.9 per cent sodium chloride solution. Some of these acid extracts were neutralized with sodium hydroxide, their reaction adjusted to pH 4.5, passed through Berkefeld filters and used directly. After excluding ten animals which at autopsy were found to be pregnant or in which accessory suprarenals were present or in which distemper,

TABLE 2

CAT NUMBER	SEX	AGE	SOLUTION INJECTED	PERIOD OF SURVIVAL
				days
109	M	4	0.9% NaCl	9.5
115	M	4	0.9% NaCl	16.0+
117	F	3	0.9% NaCl	12.0+
119	F	3	0.9% NaCl	21.0+
121	M	2	0.9% NaCl	13.0
124	F	2	0.9% NaCl	16.0+
125	M	3	0.9% NaCl	14.8
126	F	2	0.9% NaCl	19.5
222	M	3	0.9% NaCl + 0.01% $NaHCO_3$	11.3
225	M	2	0.9% NaCl + 0.01% $NaHCO_3$	7.3
226	F	1	0.9% NaCl + 0.01% $NaHCO_3$	14.2
228	M	4	0.9% NaCl + 0.01% $NaHCO_3$	30.8
231	F	3	0.9% NaCl + 0.01% $NaHCO_3$	10.8
232	M	4	0.9% NaCl + 0.01% $NaHCO_3$	16.0
234	F	4	0.9% NaCl + 0.01% $NaHCO_3$	20.1
235	F	4	0.9% NaCl + 0.01% $NaHCO_3$	7.5

bronchopneumonia and wound infection might have played a rôle, we have 10 "uncomplicated" experiments. These are summarized in table 1.

The average duration of life of these cats was approximately 12 days, or more than twice as long as the untreated control animals. These results at first glance would seem to indicate a distinct prolongation of life by the use of cortical extracts, but as pointed out above all the acid extracts were injected with salt solution and the salt plus water may have influenced the blood concentration and increased the excretion of nitrogenous waste products by diuresis. In order to decide whether it was the salt solution or the extract that prolonged life we studied a series of suprarenalectomized cats injected daily with 0.9 per cent salt solution intraperitoneally.

THE EFFECT OF 0.9 PER CENT SALT SOLUTION ON THE DURATION OF LIFE AFTER SUPRARENALECTOMY. In a few experiments 25 cc. of salt solution were injected intraperitoneally each morning and evening. In most of the experiments 50 cc. were injected once daily because of the possibility that the extra handling of the cats overbalanced the advantage of the more frequent injections of smaller amounts. After excluding 14 of the experiments in which salt solution was injected, because of pregnancy, accessory suprarenals, distemper, pneumonia or other infection, there were 16 "uncomplicated" experiments, in 8 of which 50 cc. of 0.9 per cent sodium chloride solution alone was used, while the remaining 8 received the same amount of salt solution containing 0.01 per cent sodium bicarbonate. The data of these experiments are given in table 2.

The minimum survival period was 7.3 days and the maximum, 30.8 days. The mean and average were 14.5 and 15 days respectively, that is, about three times as long as the controls and distinctly longer than those injected with the various cortical extracts containing salt solution.

A particularly interesting animal (cat 112), although eliminated from the table because an accessory suprarenal weighing 0.026 gram was found at autopsy, is of sufficient interest to report in detail. The protocol follows.

Cat 112 Young adult. Female.
5–20–25. Removed R and L suprarenals (weight 0.184 gram and 0.188 gram, respectively). 9:55–10:15 a.m. Recent lactation.
5–21–25 Bandage slipped and upper part of wound exposed. Injected 20 cc. 0.9 per cent NaCl twice daily.
5–22–25. Condition fair.
5–23–25. Eating well (salmon and meat).
6– 2–25. Discontinued saline injections. Eating full ration—condition excellent.
6–14–25. Shedding fur, active, condition good. Weight 2507 grams.
6–28–25. Not eating.
6–29–25. Not eating. Conjunctivitis, crying. Began injections of 20 cc. saline twice daily again.
6–30–25. Looks better, more active. Eating again.
7– 2–25. Discontinued injections—eating little meat. Eyes watery and nasal discharge.
7– 3–25. Not eating, weak and dull.
7– 4–25. Looks very bad, eyes closed; nose crusted over. Cleaned off. Shedding fur, not eating. Commenced saline injections.
7– 5–25. Looks somewhat better. Pus discharging from eyes and nose. Not eating.
7– 7–25. Still not eating but looks better.
7– 9–25. Ate little meat—first food since 7–3–25. Looks much better.
7–10–25. Distemper practically cleared up. Ate some meat.
7–11–25. Much better—ate all meat. Weight, 2227 grams. Discontinued saline injections.
7–16–25. Condition good—eats full ration.
7–19–25. Not eating.
7–22–25. Not eating—weight 2027 grams. Purulent discharge from eyes. Began injections again, 25 cc. twice daily.

7–24–25. Is eating again. Weight 2050 grams. More active.
7–26–25. Ate double ration—condition good, eyes clean.
7–28–25. Continues in good shape with ravenous appetite, weight 2130 grams.
7–29–25. Stopped injections for 4th time.
8– 6–25. Bright and active—has ravenous appetite. Clean but thin. Weight 2079 grams.
8–18–25. Condition excellent, weight 2340 grams.
8–27–25. Continues in good condition, eyes moist. Weight 2450 grams
9– 8–25. Covered with fleas—sprayed with kerosene. Removed impacted feces from rectum, possible intestinal stasis; very dull—marked asthenia. Injected saline again. Weight 2472 grams.
9– 9–25 Did not eat. Is very sick. Given castor oil. Injected saline solution.
9–10–25. Died last night. Weight 2195 grams. Autopsy at 9 a.m. *Thyroid:* Lobes small, pale amber, translucent. *Thymus:* Not visible in gross. *Lymph glands:* Mediastinal and retroperitoneal groups large. *Lungs:* Normal. *Heart:* Small—in systole. *Spleen:* Small. *Pancreas:* Pale. *Liver:* Small, flaccid, normal color. *Stomach:* Contains small amount of material and oil (castor oil). *Kidneys:* Normal. *Suprarenals:* Sites clean; a large accessory just below junction inferior cava and left renal vein, about 2.5 mm. in diameter, weight 0.026 gram. *Ovaries:* Small. Entire *colon* distended with dry impacted feces. Perforating ulcer of *rectum* at rim of pelvis; also ulceration in several other places. Extensive periproctitis. Slight amount of omental fat.

Major cause of death: Atony and distention of colon from impacted feces—all probably manifestations of the end stage of chronic suprarenal insufficiency.

This animal lived 112 days following suprarenalectomy. Three times during the course of the experiment salt solution injections were discontinued and each time the cat's condition became worse after varying intervals, as shown by the shedding of fur, the appearance of a nasal and conjunctival discharge and finally by anorexia and asthenia. When the salt solution injections were resumed she began to eat, became more active and the conjunctivitis cleared up. After discontinuing salt solution for the fourth time she went down rapidly. Further salt solution injections did not revive her. In this animal the well-known cycles of improvement and depression which follow sufficient suprarenal injury are greatly exaggerated, probably in part because of the function of the accessory suprarenal together with the administration of salt solution. It illustrates clearly that salt solution in one way or another can markedly prolong life after high grade suprarenal insufficiency and to a less extent after complete insufficiency.

These experiments eliminate the cortical extracts as having any important influence on the duration of life but do not indicate whether the prolongation of life was due to the sodium chloride or to the water or to their combined action.

We attempted to answer this question by injecting the same amount of salt solution in a smaller volume of water, namely, 10 cc. of a 4.5 per cent

NaCl solution once daily. This was carried out in 4 suprarenalectomized cats. The survival periods were 4.5, 4.5, 7.5 and 2.2 days respectively. The average survival period was 4.7 days and, therefore, shorter than that obtained in our control series.

These four experiments were considered sufficient to indicate that water plays a very important rôle in the prolongation of life. It may be asked why the next step was not the administration of water by stomach tube. We felt that more harm than good would result because of the struggling that necessarily results from that procedure. Suprarenalectomized animals are particularly sensitive to exertion because of the cardio-vascular asthenia and every precaution must be taken to prevent unnecessary cardiac strain.

THE EFFECT OF RINGER'S SOLUTION, SODIUM GLYCEROL-PHOSPHATE AND SODIUM ACETATE ON THE DURATION OF LIFE AFTER SUPRARENALECTOMY.

A. Ringer's solution. While these experiments were in progress Stewart and Rogoff (6) published data showing that dogs suprarenalectomized in two stages and injected with Ringer's solution containing glucose lived 3 or more times as long as their untreated controls. We have also found that plain Ringer's solution prolonged life about as well as salt solution. Indeed, there was a possibility from our first experiments that it might be somewhat better than salt solution, and on account of this possibility we carried out additional experiments using Ringer's solution modified *1*, by omitting sodium bicarbonate; *2*, by omitting calcium chloride and sodium bicarbonate, and *3*, by omitting potassium chloride and sodium bicarbonate. Unfortunately, during this series of experiments with Ringer's solution we had an outbreak of severe distemper and none of the results we feel are entirely free from this factor. The results are tabulated below

Of the 8 animals injected daily with 50 cc. of Ringer's solution, 4 were excluded because of pregnancy, accessory suprarenals and distemper. The remaining 4 we believe were also not entirely free from distemper.

Of the 13 cats injected with Ringer's solution minus sodium bicarbonate, 7 were excluded because they developed severe distemper a few days after operation and the 6 included were probably affected. Of the 7 injected with Ringer's minus potassium chloride and sodium bicarbonate, 2 were excluded—one because of an accessory and the other because it was accidentally exposed to illuminating gas. Of the 15 injected with Ringer's solution minus calcium chloride and sodium bicarbonate, 5 were excluded. Of these, 2 were pregnant, 1 had an accessory, 1 had acute pneumonia and 1 was complicated by the shock and trauma of a difficult operation.

The variously modified Ringer's solutions gave results so nearly alike as far as can be judged from this crude method of analysis, that they may be treated as a single group. In all, 43 animals were used, of which 25 have been included in the table. As pointed out above, we feel that the figures

for duration of life are minimal since all were more or less exposed to a severe distemper epidemic. The maximum and minimum duration of life after suprarenalectomy was 34.2 and 6.8 days respectively. The mean was 11.0 and the average, 13.1 days.

On the whole, these results are similar, though slightly lower than those obtained with salt solution and we feel that the favorable effect of

TABLE 3

CAT NUMBER	SEX	AGE	SOLUTION INJECTED	PERIOD OF SURVIVAL
				days
179	M	2	Ringer's	11
181	F	3	Ringer's	11.2
185	F	3	Ringer's	7.2
186	F	2	Ringer's	34.2
156	F	2	Ringer's minus $NaHCO_3$	11.8
157	F	4	Ringer's minus $NaHCO_3$	20.8
158	M	3	Ringer's minus $NaHCO_3$	9.8
161	F	1	Ringer's minus $NaHCO_3$	7.2
207	M	1	Ringer's minus $NaHCO_3$	11.5
211	M	2	Ringer's minus $NaHCO_3$	13.0
171	F	2	Ringer's minus $NaHCO_3$ and KCl	7.8
174	F	2	Ringer's minus $NaHCO_3$ and KCl	9.5
175	F	2	Ringer's minus $NaHCO_3$ and KCl	17.5
176	F	2	Ringer's minus $NaHCO_3$ and KCl	9.8
177	M	4	Ringer's minus $NaHCO_3$ and KCl	10.8
163	F	2	Ringer's minus $NaHCO_3$ and $CaCl_2$	11.0
166	F	2	Ringer's minus $NaHCO_3$ and $CaCl_2$	9.0
167	F	2	Ringer's minus $NaHCO_3$ and $CaCl_2$	31.2
168	M	2	Ringer's minus $NaHCO_3$ and $CaCl_2$	8.5
169	F	2	Ringer's minus $NaHCO_3$ and $CaCl_2$	22.8
170	M	4	Ringer's minus $NaHCO_3$ and $CaCl_2$	15.2
199	F	3	Ringer's minus $NaHCO_3$ and $CaCl_2$	12.8
200	M	3	Ringer's minus $NaHCO_3$ and $CaCl_2$	7.3
205	M	3	Ringer's minus $NaHCO_3$ and $CaCl_2$	6.8
206	M	2	Ringer's minus $NaHCO_3$ and $CaCl_2$	10.5

Ringer's solution is of the same nature as that caused by salt solution. The other salts in Ringer's solution appear to have no particular significance.

B. Sodium glycerol-phosphate. Since salt solution is clearly of great importance in prolonging the life of suprarenalectomized animals it seemed possible that in addition to its diuretic effects there might be some specific action either of the sodium or chlorine ions and in order to obtain some

information on this point we first tried an isotonic sodium glycerol-phosphate solution. This was made by neutralizing a commercial glycerol phosphoric acid solution with sodium hydroxide to pH-7 and diluting it. Fifty cubic centimeters of this solution were injected intraperitoneally each day. Of the 8 cats used, 2 were excluded because of pregnancy and one because an accessory suprarenal was found at autopsy. The results of the remaining 5 are summarized in table 4.

The maximum and minimum duration of life were 11.3 and 8.5 days respectively, while the mean and average were 10.5 and 10.2 days. The prolongation of life is about twice that of our controls, but distinctly below

TABLE 4

CAT NUMBER	SEX	AGE	PERIOD OF SURVIVAL
			days
148	F	2	11.3
149	M	3	8.5
150	M	3	11.3
151	F	2	10.5
153	M	4	9.2

TABLE 5

CAT NUMBER	SEX	AGE	PERIOD OF SURVIVAL
			days
241	M	3	19.8
248	F	2	8.5
249	F	2	20.1
250	M	4	10.5
252	F	1	2.5
254	F	4	13.8
255	M	4	19.0
256	M	2	8.0

that obtained with salt solution and Ringer's solution. It is possible that the large amount of phosphate contained in 50 cc. of isotonic glycerol-phosphate solution even though the phosphate is liberated gradually might have an injurious action.

C. Sodium acetate. Salts of sodium, other than sodium chloride, available for such injections are limited, and in order to extend the series as much as possible sodium acetate was tried. It was realized that an injection of sodium acetate might tend to cause alkalosis. Fifty cubic centimeters of an isotonic solution (1.2 per cent) were injected daily into 13 suprarenalectomized cats. Five of these have been excluded. Of these 5, 2 were pregnant, 2 died from pneumonia and 1 had wound infection.

The remaining 8 while not entirely free from complications are given in Table 5.

The maximum survival period was 20.1 days and the minimum was unusually low—2.5 days. We could ascertain no cause for death in this case other than acute suprarenal insufficiency and therefore have included it. This lowers the mean and average duration of life to 12.1 and 12.8 days respectively. If the low result were excluded the average would be 14.3 days and in spite of the relatively few experiments we feel that 14.3 days is a fairer average than the actual one. The outcome is distinctly better than with sodium glycerol-phosphate and probably quite as good as with salt solution and Ringer's solution. Chlorine, therefore, seems not to be an important factor in the prolongation of life, even though, as several authors have shown, the blood chlorides are reduced after suprarenalectomy. The relative importance of sodium and water in relation to the survival period was still undetermined. It seemed possible that additional data

TABLE 6

CAT NUMBER	SEX	AGE	PERIOD OF SURVIVAL
			days
128	M	2	2.8
131	F	3	9.0
132	M	3	6.8
133	F	1	6.0
134	F	2	15.0
135	M	3	7.5
137	F	4	5.0
138	F	1	4.5

on this point might be obtained from a study of the effects of glucose and glycerol solutions.

THE EFFECT OF GLUCOSE AND GLYCEROL SOLUTIONS ON THE DURATION OF LIFE AFTER SUPRARENALECTOMY. *A. Glucose.* Twenty cubic centimeters of an isotonic solution of glucose were injected twice daily intraperitoneally into 10 cats. Two of these have been excluded, one because an accessory suprarenal was found at autopsy and the other because of pregnancy. The remaining 8 are summarized in Table 6.

The maximum and minimum duration of life were 15.0 and 2.8 days respectively, while the mean and average were 6.4 and 7.1 days.

B. Glycerol. Similar experiments were made with an isotonic solution of glycerol injected intraperitoneally in 20 cc. doses twice daily. Of the 6 cats used two were excluded, one because an accessory was found at autopsy and the other because of wound infection. The remaining four lived 12.5, 6.5, 6.3 and 2.5 days respectively, an average of 6.9 days.

Glucose and glycerol solutions, therefore, appear to be of about equal value as regards prolongation of life. Life was prolonged on the average of about 2 days more than the controls but only about one-fourth that where salt solution was used.

Discussion. Of the 167 cats used in this study only 101 were considered sufficiently free from possible complicating factors to be included in the tables of analysis. Of the 66 excluded 20 were pregnant and 11 had accessory suprarenal glands verified by histological examination. This is a higher percentage of accessories than those given in the literature reports.

One of the most striking features of this study was that no animal in which accessories were not found lived longer than 34 days. This is in sharp contrast with rats and rabbits where, under our conditions, about 40 per cent of rabbits and 60 per cent of rats will survive more than a month in good condition. The more frequent occurrence of accessory suprarenals in rabbits and rats we believe will not entirely explain the differences in the period of survival. A factor of considerable importance in cats may be the difference in the diets which permits a greater retention of nitrogenous waste products normally excreted by the kidneys.

In addition to these factors there are in any given species (dog, cat, rat, rabbit) marked individual differences in the duration of life which cannot be fully accounted for on the basis of accessories, diet, tissue dehydration, infection, injury to the sympathetic nervous system or to general trauma of operation. This difference, it seems to us, may depend in part on the variations in the amount of the suprarenal influence present in the various tissues at the time of operation and may be comparable to the variations seen in the time of onset of tetany after parathyroidectomy or in the fall of metabolism after thyroidectomy.

Our control animals have lived on the average 5.3 days, which is considerably longer than any series previously reported. This difference we ascribe entirely to the rapid, simple operation. As regards the general behavior and final outcome after suprarenalectomy in cats our studies in general confirm those of others.

The most important observation concerns the effect of sodium salts and water in prolonging life. Others, notably Soddu (7), Marshall and Davis (3) on cats, and more recently Stewart and Rogoff (6) and Banting and Gairns (8) on dogs, have noticed this effect. Our series of 18 cats in which nothing but the routine diet was given lived on an average of 5.3 days, whereas 16 cats which received in addition 50 cc. of physiological salt solution daily lived on an average of 15.0 days; 25 which received 50 cc. of Ringer's solution daily lived on an average of 13.1 days; and 8 which received 50 cc. of a 1.2 per cent sodium acetate solution lived on an average of 12.8 days.

Of several possibilities which these data suggest, two may be discussed

here. Was the prolongation of life due mainly to the diuresis, or was there in addition some specific effect of the sodium ion? As pointed out by Marshall and Davis and confirmed by all subsequent observers, there is a marked retention of nitrogenous waste products in the blood of suprarenalectomized cats which comes on early while the animal is in good condition, and is clearly not terminal. In the cat there are also gross lesions in the kidneys which suggest that the retention is due to renal injury.

Our studies indicate that Ringer's solution has no advantage over physiological salt solution and these in turn are apparently no better than sodium acetate. These observations indicate that chlorine is not an important factor even though there is regularly a decrease in blood chlorides after suprarenalectomy.

The same amount of sodium chloride given as a 4.5 per cent solution actually shortens life. This would indicate that water is indispensable and further supports the diuresis hypothesis of the cause of the prolongation of life since hypertonic salt solution by hastening dehydration reduces the animal's means of flushing out waste products through the kidney.

On the other hand an isotonic glucose solution, while it is an excellent diuretic, yet when given with the same amount of water as was given when physiological salt solution was used, fails to prolong life materially. Such solutions of glucose while amounting only to the administration of 2.7 grams of glucose daily may be injurious because they bring about a greater excretion of water than is compensated for by the amount given.

The possibility that sodium salts have a specific action due to the sodium ion must be considered. The observations (9) of one of us who found a significant reduction in the blood sodium of cats after suprarenalectomy could be used in support of such a view. On the other hand a loss of base accompanies both acidosis and diuresis, and the existence of acidosis after suprarenalectomy has recently been reported by Swingle and Eisenman (10). Much work remains to be done before a final statement is possible regarding the rôle of diuresis alone or plus a specific action of sodium in the prolongation of life. Obviously water is necessary whether the prolongation of life is due to better renal function or to a specific action of sodium. The difficulties of giving water by stomach tube to suprarenalectomized cats are very great and in our experience life has been shortened because of the struggling. Nevertheless some means must be devised for accomplishing it in order either to eliminate or establish the importance of sodium as a specific factor in the prolongation of life.

While the administration of various sodium salt solutions will prolong the life of suprarenalectomized cats about three times that of untreated controls, it is obvious that their action is only palliative. All our animals have ultimately died of typical suprarenal insufficiency.

The obvious renal injury, the nitrogen retention, the tissue dehydration, the loss of blood chlorides and sodium, the hypersensitiveness to poisons, the lymphoid hyperplasia, the increased thyroid activity, the cardio-vascular asthenia, the gastro-intestinal lesions, and many other manifestations of suprarenalectomy, all seem to be individual details of a more fundamental disturbance in nutrition in which the sympathetic nervous system is primarily concerned.

SUMMARY

The data of the 101 "uncomplicated" experiments used in this study are summarized in the following table:

TABLE 7

SOLUTION INJECTED	NUMBER OF ANIMALS	PERIOD OF SURVIVAL IN DAYS			
		Maximum	Minimum	Mean	Average
1. Controls	18	12.0	2.0	5.2	5.3
2. Thyroidectomized controls	3	6.1	5.0	6.0	5.7
3. Cortical extracts	10	17.0	8.5	11.8	11.9
4. 0.9 per cent NaCl	16	30.8	7.3	14.5	15.0
5. 4.5 per cent NaCl	4	7.5	2.2	4.5	4.7
6. Ringer's	25	34.2	6.8	11.0	13.1
7. Sodium glycerol-phosphate	5	11.3	8.5	10.5	10.2
8. Sodium acetate	8	20.1	2.5	12.1	12.8
9. Glucose	8	15.0	2.8	6.4	7.1
10. Glycerol	4	12.5	2.5	6.4	6.9

Control cats from which both suprarenals have been removed at one operation have survived on an average of 5.3 days. The administration of physiological salt solution, Ringer's solution and isotonic sodium acetate solution have increased the duration of life about 3 times that of controls. More concentrated solutions of sodium chloride definitely shorten life. Isotonic glucose and glycerol solutions have only a very slight life-prolonging effect. One may conclude from these studies that diuresis is one of the important factors determining the duration of life. When the loss of water by diuresis is compensated for by an additional intake, life is prolonged, and if not, life is shortened. There is some indication that the loss of sodium is more specific than can be accounted for as a result of a possible acidosis.

It is believed that all the manifestations of suprarenalectomy thus far observed are the individual details of a more fundamental and as yet unknown disturbance in nutrition in which the sympathetic nervous system is primarily concerned.

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THE RELIEF OF EXPERIMENTAL ILEUS BY SPINAL ANESTHESIA

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Although various hypotheses have been formulated to explain paralytic ileus, few of them have had a physiological basis. Several common states, both surgical and physiological, present a striking analogy to the severe ileus which is observed when the intra-abdominal surgical manipulations have been very extensive. Thus it is exceptional to see bowel movements at laparotomy, and occasionally even following a simple abdominal operation—as for omental hernia—the intestines fail to resume their motility, so that the patient develops the syndrome of "paralytic ileus." Allied to the intestinal paralysis observed at operation is the immediate cessation of bowel movements which occurs in patients following perforation of a gastric or a duodenal or a typhoid ulcer, and which is revealed on auscultation of the abdomen by absence of the usual sounds. The paralytic ileus of acute diffuse peritonitis is also a well-known phenomenon, and indeed, as Kelby and Ivy (1) have shown, is a definite factor in the high mortality of this condition. The sudden onset of all these types of bowel paralysis would seem to indicate that the cessation of movements is reflex in nature; and the almost imperceptible transition into what is arbitrarily called "paralytic ileus" suggests that the latter is an extension of the former, with, possibly, secondary changes in the wall of the bowel due to stagnation of the intestinal contents, intra-peritoneal suppuration, local anemia or other causes.

In laboratory animals the analogy between reflex intestinal paresis and paralytic ileus is even more striking. In dogs, rabbits and cats it is unusual to observe bowel movements after opening the abdomen, even when these have been demonstrated radiologically to be quite vigorous before operation. By watching the shaved abdomen of a fed rabbit it is possible to see bowel movements through the abdominal wall. Yet it is not possible to see these movements once the peritoneal cavity has been opened (2), (3), (4). Bayliss and Starling (5) found on opening the abdomen of a dog in a warm saline bath, that the intestines were collapsed and absolutely motionless, and that it was necessary to cut the splanchnics or to destroy the spinal cord or to remove the abdominal ganglia before movements

occurred. They also found that fifteen to thirty minutes had to elapse after division of the nerves before the previously motionless intestines gradually became more active and rhythmic bowel movements occurred. Local electrical or mechanical stimulation was either without effect, or merely caused a contraction limited to the stimulated spot, and even distention of the gut with a rubber balloon containing compressed air evoked no response until the thoracico lumbar nerve pathway had been destroyed.

Meltzer and Auer (3) also reported that dissecting the skin over the abdomen caused reflex inhibition of peristalsis in rabbits. It is possible also that the irritation due to the sutures approximating the cut edges of the parietal peritoneum after laparotomy in the human subject might cause an inhibition in some patients which, if sufficiently complete and prolonged, would be classed as paralytic ileus.

Since no relationship can be demonstrated between the fall in blood pressure after splanchnic section and the resumption of bowel movements, it is evident that this type of intestinal paresis must be of the nature of an inhibition, efferent pathway being the splanchnics, and it occurred to us that paralytic ileus might, in the earlier stages at any rate, be due to a similar cause. This hypothesis would explain the occasional development of ileus after severe injury to a limb, after a trifling abdominal operation, or even after a severe nervous shock.

In the light of these considerations, it should be possible to cure paralytic ileus either by *a*, blocking conduction through the splanchnics; or *b*, doing away with the irritant stimuli. We considered that both these conditions could be established by injecting sufficient novocaine into the spinal canal.[1] It is common knowledge that this procedure will induce surgical anesthesia of the whole peritoneal cavity, and we hoped would also prevent efferent stimuli from reaching the bowel *via* the splanchnics, by blocking the rami communicantes.

Accordingly we investigated the effect of spinal anesthesia on the following conditions in dogs:

a. The intestinal paresis observed during laparotomy.

b. The ileus produced by injecting a solution of iodine intraperitoneally (6).

c. The ileus induced by such intra-abdominal manipulation as rubbing the parietal and visceral peritoneum with gauze or by moderately rough stripping of the intestines between the fingers (7).

EXPERIMENTAL. Five typical protocols are presented:

Protocol 1. May 22. Male collie; 21 kilos. Fed. At 12:45 50 mgm. novocaine in 2 cc. water were injected intraspinally at the level of the lumbosacral articulation.

[1] When these experiments were undertaken we were not aware of the work of Wagner *vide infra*.

Priapism occurred, and the animal defecated immediately. Almost at once there ensued paraplegia and insensitivity to pin pricks from the mid-thoracic region down. At 1:00 the injection of novocaine was repeated. At 1:15 laparotomy was performed, the operation being painless. The intestines were exposed freely to the air, and were handled as though the animal were under a general anesthetic. In spite of this there were frequent typical pendular movements in the small bowel, with an occasional peristaltic wave. The stomach appeared quiescent. The animal was sewn up and made an uninterrupted recovery. At 11:00 a.m., May 31, the animal was given a barium meal consisting of 5 heaped tablespoonfuls of $BaSO_4$ in 300 cc. buttermilk. At 12:00 o'clock the animals was placed in front of a fluoroscope, and it was noted that the small bowel was filled with shadows showing lively segmental and occasional peristaltic movements. Movements continued up to 2:00 p.m., when 20 cc. of 2 per cent iodine in 2 per cent potassium iodide were injected intraperitoneally. At 2:10 the animal was again screened. Intestinal movements had stopped almost completely, there being only an occasional pendular or peristaltic movement. At 3:00 there was almost complete inhibition of all movements. Twenty milligrams of novocaine in 2 cc. water were now given intraspinally. There was good anesthesia of the posterior half of the body although complete paraplegia did not result, the animal being ataxic in the hind limbs. At 3:45 the animal was screened. Bowel movements were excellent. There were lively segmentation and peristalsis, these movements being definitely exaggerated as compared with the preliminary control examination. The stomach showed but slight peristalsis.

Next day at 11:00 a.m. the animal showed many shadows in the transverse and descending colon and rectum. A glycerine enema was given and several hard lumps of feces consisting chiefly of $BaSO_4$ were evacuated. At 12:05 a barium meal was given. At 12:10 fluoroscopic examination showed the stomach to be full and the duodenal cap well defined. At frequent intervals gushes of barium mixture passed from the duodenal cap into the intestine for a distance of several inches. At 12:15 segmenting shadows began to appear in the small bowel. At 1:00 there were occasional ejections of barium into the jejunum, but the head of the meal progressed slowly and there was evidently some inhibition of bowel activity. At 2:15 the whole belly was full of shadows showing active segmenting movements and occasional peristalsis. At 2:30 20 cc. of 2 per cent iodine in potassium iodide were injected intra-abdominally. At 2:35 movements were practically absent and no food was issuing from the pylorus. The bowel was dilated and adjacent masses of the barium shadow were fused together. At 2:50 the picture was the same, the stomach being a motionless mass, the shadows in the small bowel being disposed as a number of large masses, between which the gut was empty. These showed here and there an occasional segmental movement. At 3:07 60 mgm. novocaine in 3 cc. water were given intraspinally and complete paraplegia ensued. At 3:15 there were vigorous peristaltic movements in the stomach and in the whole of the small bowel. Pendular and peristaltic movements were often seen, and large gushes of material were being ejected from the stomach. Fluoroscopic observations were continued for more than an hour, the intestinal movements being definitely exaggerated throughout this period. At 4:30 laparotomy was performed, the operation being painless. When the abdomen was opened much serosanguinous fluid poured out and the great omentum was found to be adherent to the parietal peritoneum, forming a large walled-off cavity into which the injections of iodine had been made. The intestines were reddened and matted together with partially organized plastic exudate. Segmental movements were present all over the small bowel and gentle handling of the gut elicited peristalsis. The stomach was quiescent.

The animal was chloroformed.

Protocol 2. June 8. Small black dog of about 8 kilos. At 2:30 he ate a barium meal with relish. At 2:50 the animal was fluoroscoped, the stomach and intestines showing excellent movements. At 4:00 the belly was still full of moving shadows. At 4:05, 6 cc. 2 per cent iodine in potassium iodide were injected intraperitoneally. At 4:10 the stomach was seen as a motionless shadow with an indentation indicating a tonus ring in the mid-gastric region. The pyloric portion of the greater curvature also showed motionless tonic constrictions. Food left some parts of the small intestine to collect in others, the result being the formation of several dilated motionless loops. At 4:25 the animal vomited a few cubic centimeters of curdled buttermilk and barium. The intestinal movements were practically completely inhibited. At 4:30, 1 cc. spinal fluid was withdrawn (to make certain the spinal canal had been entered) and 30 mgm. novocaine in 2 per cent solution were injected. Paralysis of the hind limbs and defecation followed. At 4:45 the animal was again screened, when it was seen that the movements were exaggerated and that peristalsis was often present. The bowel was in a constant turmoil of activity and what food was left in the stomach was being squirted at short intervals through the duodenal loop into the jejunum. Very strong peristaltic waves were seen in the stomach and small intestine. At 5:00 the animal became dyspneic and 5:15 it stopped breathing, probably due to the effect of novocaine on the respiratory centre. We think we observed peristaltic rush at this time.

At autopsy the peritoneal cavity contained about three-quarters litre of serosanguinous fluid. The parietal and visceral peritoneum was red and injested. The intestines were red, and at about the middle of the jejunum the peritoneal and muscular coats were stained with iodine.

Protocol 3. June 2, 1926. Irish terrier weighing about 12 kilos. At 1:30 the animal was given a barium meal. At 3:50 there were many segmenting shadows in the small bowel and these often showed typical peristalsis. At 4:00 the movements were excellent and a large part of the small intestine contained barium. At 4:10, 10 cc. of 2 per cent iodine in potassium iodide were given intraperitoneally. At 4:20 there was practically complete inhibition of all movements. The intestines were dilated in several places in which barium was collecting. At 4:30 3 cc. of spinal fluid were removed and 2 cc. of 2 per cent novocaine were injected. There was almost immediate posterior paraplegia. At 5:05 brisk movements were seen in the stomach and small intestine, but at 5:10 the movements were not as marked. At 5:20 and again at 5:30 they were brisk again. Shadows were seen in the colon showing peristalsis, and possibly antiperistalsis, though we were not certain of this point.

The general condition of the animal after the administration of the novocaine is of interest. At 4:45 it was weak, apathetic and breathing rapidly. It gradually improved so that at 5:30, although it showed the usual paraplegia, the upper half of the body was normal and the animal was mentally alert.

Protocol 4. June 8. A wire-haired terrier of about 5 kilos, received a barium meal at 1:00 p.m. At 2:10 good movements were seen under the x-ray and there was fair filling of the intestine. At 3:30 excellent movements were visible all over the abdomen. At 4:15 5 cc. of 2 per cent iodine in potassium iodide were injected intraperitoneally. Typical inhibition resulted. At 5:35 spinal puncture was performed. Though we failed to get spinal fluid we assumed we were in the spinal canal when we felt the sudden "give" of the needle. Ten milligrams novocaine in 2 per cent solution were administered. The animal defecated and typical paraplegia followed. At 5:35 segmental movements began to appear, but the effect of the injection was not so marked as usual. At 6:00 novocaine was again injected, this time with much more success, for at 6:05 the stomach began to empty itself at a remarkable rate.

A tonus ring appeared well to the left, near the fundus and deep peristaltic waves were seen being propagated rapidly over the stomach. Vigorous segmental and peristaltic movements were seen in the intestine. At 8:45 the animal, though somewhat anesthetic, could walk in a stiff manner. Most of the meal was in the colon; the small intestine was still showing active movement.

Protocol 5. August 17. Large collie. Barium meal at 10 a.m. At 11:30 the belly was seen to be full of actively moving shadows. At 12:00 noon, under ether anesthesia laparotomy was performed without aseptic precautions. The whole parietal and visceral peritoneum was rubbed vigorously with gauze and the stomach and intestines were tugged and moderately stripped between the fingers. At 1:45 the incision was closed and rapid recovery ensued. At 4 p.m. an occasional segmental movement was observed in the region of the ileum, but otherwise the gut was quiescent. At 4:15 4 cc. of spinal fluid were withdrawn and 4 cc. of 2 per cent novocaine were injected. The animal defecated and immediately became paraplegic after which, at 4:30, it was screened. Bowel movements were seen to have returned and *to be more vigorous than before operation.* There was rapid segmentation and peristalsis in the whole small bowel. The stomach was contracted down, and frequent movements were visible with gushes of barium shadow being squirted through the pylorus.

Next day at 12:30 the bowel was empty and the animal was given barium meal by stomach tube. Although the stomach and intestines on fluoroscopic examination showed many movements the progress of the meal was very slow.

The animal was chloroformed.

Discussion. It is difficult to generalize concerning reflex inhibition of bowel movements because apparently different animals do not react alike. For example, Meltzer and Auer (3) found in the rabbit that the intestinal paresis consequent upon opening the abdomen persisted even after preliminary section of the cord in the region of the third dorsal vertebra, whereas Cannon and Murphy (7), (8) observed that trauma of the testicles in the anesthetized cat caused marked delay in the emptying time of the stomach, as well as inhibition of bowel movements, but that a similar effect did not follow this trauma after section of the splanchnic nerves. On the other hand, they found that splanchnotomy did not influence the inhibition of the bowel movements induced by rough handling of the gut.

Such considerations call for caution in the interpretation of the results presented in this paper. These show that spinal anesthesia in the dog abolishes the inhibition of bowel movements brought about by laparotomy, or by the intraperitoneal injection of iodine, or by traumatizing the bowel and peritoneum, and it is of practical importance to note that Wagner (9) has observed that spinal anesthesia is promptly curative in cases of paralytic ileus in man, a fact which others have since confirmed (10).

These observations make it seem very probable that paralytic ileus is essentially reflex in nature and that the chemical changes which have been described in the blood are the result and not the cause of the ileus. It is notoriously very difficult to cause persistent paralytic ileus in dogs by severe intra-abdominal traumatism, although such treatment would all too

certainly bring on this condition in man. This difference in response is probably not fundamental, but is one of degree only. The stimulus which in man, with his highly organized nervous system, causes paralytic ileus, in the dog causes merely severe inhibition of bowel movements.

Assuming that our results as obtained in the dog hold good also for man, paralytic ileus as met with in the clinic must be of the nature of reflex inhibition, dependent, therefore, on the integrity of the reflex arc. Such a conception implies that the paralysis of bowel movements observed at laparotomy is a *physiological ileus* which if unrelieved or augmented by other irritant stimuli merges into *paralytic ileus*. No other conception is in harmony with the observation of Wagner that spinal anesthesia promptly restores bowel movements in cases of paralytic ileus.

Just how novocaine administered intraspinally acts in curing ileus is difficult to explain with certainty. It induces surgical anesthesia of the lower half of the body, thus stopping afferent stimuli; and as judged by its action on blood pressure it blocks the splanchnics (11). The temporary paraplegia observed in our animals testifies also to its paralyzing action on voluntary motor nerves. It appears, therefore, that in the intraspinal administration of novocaine we possess a method of temporarily blocking transmission through the spinal cord.

SUMMARY

The reflex paralysis of bowel movements induced in dogs by laparotomy, or by the intraperitoneal injection of iodine, or by severe intra-abdominal traumatism, is promptly abolished by spinal anesthesia with novocaine. The possible mechanism of this action is discussed. The conception of paralytic ileus as a reflex inhibition of bowel movements which can be abolished by spinal anesthesia is introduced.

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IRIS MOVEMENTS IN BLIND MICE

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The classic researches of Arnold (1841) and Brown-Sequard (1847) first brought to light the interesting fact that movements of the vertebrate iris might be elicited by direct luminary stimulation. It is now well known that the completely isolated eel iris responds by contraction or dilatation to stimuli of light or darkness. The eel iris is capable of reacting to light several weeks after enucleation in eyes left intact.

The most pronounced reactions seem to be found at the lower end of the vertebrate series. These responses become weaker as one passes higher in the scale. This apparent gradual diminution in the power of the iris, when isolated from the central nervous system, to respond to local stimulation by light becomes so great at the upper end of the phylum that there has been much doubt expressed as to its existence in mammals.

In the case of mammals some investigators are inclined to believe that the iris is in some fashion dependent upon the proper functioning of the retina and even use the ability of the iris to dilate and contract as a criterion of vision.

Another school believes that in the iris of mammals as well as lower forms, there is a weak independent-effector response following local stimulation under our experimental conditions and that normally throughout the whole phylum the vertebrate iris behaves entirely independent of visual ability. The critics of this view claim that even in the most successful mammalian experiments, the results obtained can be explained as a nervous reflex retained in the eye as a whole for a short time following enucleation. Indeed, the percentage of cases in which any response of the iris to photo-stimulation has been found in enucleated eyes of mammals is very low.

THEORY. However, we know that the mammalian eye is an extremely delicate, highly complex mechanism in its ensemble. It may be that we are unable to isolate particular systems of which it is composed without greatly impairing the working power of the rest by our brutal methods. Perhaps it is impossible to separate the retina and the iris in mammalian eyes without such injury to the latter as would prevent its subsequent response to stimulation by light. In extirpation experiments one undoubtedly changes the

pressure, nutrient nature of the liquid medium and cuts off the normal means of respiration of the iris, to say nothing of probable mechanical injuries resulting from surgical technique.

But how is it possible without disturbing respiration, pressure and nutrition to eliminate the visual function of the retina and maintain the iris unaffected for our observations?

Human clinical material in which the visual power is lost is usually of a pathological nature. We cannot expect to find in such material the iris perfectly healthy and unaffected by any internal infection.

MATERIAL. Several years ago, by methods of inbreeding, I isolated a pure strain of mice which never develop the sensory elements (rods) in the eye. Histological studies show no differences between such an eye and the normal eye save in the incomplete differentiation of the external layers of the retina. By a detailed series of behavior experiments I was able to establish the fact that such animals are completely devoid of vision. These mice present an iris quite normal in appearance, both gross and microscopic.

Here, then, we have an experimental animal with a visually functionless eye presenting apparently a normal iris. An examination of the photo-irritability of such material should prove interesting in comparison to the normal.

Choice of material. I have purposely avoided the use of albino material on account of its hyper-photo-sensibility which would increase the difficulties of our problem. Pigmented mice only with highly pigmented eyes have been chosen for this study. Adults of six months of age or over have been used, lest size differences should invalidate our comparisons. Pregnant females were not employed because nervous states might enter into the results.

Apparatus. For observations of the action of the iris a Zeiss binocular dissecting microscope with an electrically illuminated field was employed. In the right ocular was placed an ocular-micrometer for measuring the pupillary diameter. At the distance of the focus, one ocular-micrometer space represented 0.077 mm. actual width.

A metronome beating 100 strokes per minute was used to measure the time. Hence the time between any two successive beats was 0.6 second.

PROCEDURE. Every eye examined was subjected to 15 seconds' total darkness. The light was then flashed on and the movements of the pupil observed. An estimate of the average closure for each pupil was made. Five tests for each eye were recorded measuring the latent period and the period of contraction.

To test the greatest dilatation of the iris possible, a drop of atropin was placed in the eye under observation and the pupillary diameter measured five minutes later.

TABLE 1

INDIVIDUAL	EYE	CONDITION OF RETINA	AVERAGE CONTRACTION	TIME OF LATENT PERIOD					TIME OF CONTRACTION					DIAMETER OF PUPIL	
				1	2	3	4	5	1	2	3	4	5	Atropin	Sulfide of eserine
Gray ♀ 28	Left	Normal	1.46-0.616	0.3	0.3	0.3	0.3	0.3	3.0	3.0	3.0	3.0	3.0	2.31	0.231
Black ♂ 23	Left	Normal	1.54-0.539	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.3	3.3	3.3	2.31	0.099
Black ♂ 23	Right	Normal	1.54-0.539	0.6	0.6	0.6	0.6	0.6	3.0	3.3	3.3	3.3	3.3	2.31	0.099
Gray ♀ 12	Left	Normal	1.54-0.539	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.6	4.2	4.2	2.31	0.924
Gray ♀ 12	Right	Normal	1.54-0.616	0.6	0.6	0.6	0.6	0.6	4.2	4.2	4.2	5.6	5.6	2.31	0.924
Gray ♀ 13	Left	Normal	1.54-0.616	0.6	0.6	0.6	0.6	0.6	3.6	3.6	3.6	3.6	3.6	2.31	0.385
Gray ♀ 13	Right	Normal	1.54-0.616	0.6	0.6−	0.6	0.6	0.6	3.6	3.0	3.0	3.0	3.0	2.31	0.385
Gray ♀ 10	Left	Normal	1.54-0.693	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.0	3.0	3.0	2.31	0.154
Gray ♀ 10	Right	Normal	1.54-0.616	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.0	3.0	3.0	2.31	0.154
Chinchilla ♀ 11	Left	Normal	1.54-0.616	0.6+	0.6	0.6	0.6−	0.6	5.4	5.4	5.4	5.4	6.8	2.16	0.616
Chinchilla ♀ 11	Right	Normal	1.54-0.616	0.6+	0.6+	0.6+	0.6+	0.6+	4.8	5.4	5.4	5.4	5.4	2.16	0.616
Averages			1.53-0.602					0.57					3.73	2.28	0.417
Gray ♀ 31	Left	Rodless	1.54-0.62	2.4	2.4	2.7	2.7	2.4	2.4	2.4	2.1	2.1	1.8	2.31	0.154
Gray ♀ 31	Right	Rodless	1.54-0.62	3.0	3.3	2.7	3.0	3.0	2.4	1.5	1.5	2.1	1.8	2.31	0.154
Black ♂	Left	Rodless	2.31-1.16	3.3	3.6				6.0	6.6	Animal choked to death				
Chinchilla ♀	Left	Rodless	1.39-0.61	1.8	1.8	1.8	2.4	1.8	3.0	3.0	3.0	3.0	3.0	2.70	0.385
Chinchilla ♂	Left	Rodless	1.39-1.16	1.2	1.5	1.8	1.8	1.8	3.0	2.4	2.4	2.4	2.4	2.70	0.308
Brown ♂ 7	Left	Rodless	1.93-1.16	1.8	3.0	3.0	3.0	1.8	3.0	2.0	3.0	3.0	2.0	2.39	0.154
Brown ♂ 7	Right	Rodless	1.93-1.16	0.6	0.6	2.4	1.8	2.4	2.4	3.0	1.8	3.0	2.4	2.39	0.154
Brown ♀ 34	Left	Rodless	1.54-0.77	1.8	3.0	3.0	3.0	3.0	2.4	1.8	2.4	3.0	1.8	2.39	0.365
Brown ♀ 34	Right	Rodless	1.54-0.77	1.8	3.0	3.0	3.0	4.8	3.0	1.8	1.8	2.4	1.8	2.31	0.365
Brown ♀ 6	Left	Rodless	1.54-1.16	1.8	1.2	1.8	0.6	0.6	3.0	1.8	2.4	2.4	3.6	2.39	0.231
Brown ♀ 6	Right	Rodless	1.54-1.16	1.8	1.5	1.2	0.6	0.6	2.4	2.4	2.4	3.6	3.6	2.39	0.231
			1.65-9.40					2.18					2.56	2.43	0.250

All diameters are given in millimeters. All times are given in seconds.

After three days, in order to learn the extent of extreme pupillary contraction, a drop of sulfide of eserine was dropped into an eye of each experimental animal. This dose was strong enough to contract all parts of the body violently. After five minutes the results were recorded.

An eye from each of the experimental animals was enucleated and paraffin sections were prepared.

The results of this study are set forth in the following table.

By classifying the data according to the histological condition of the retina we find the average amount of contraction for rodless mice to be from 1.65 to 0.940 mm. for controls from 1.53 to 0.602 mm. The average period of latency for rodless eyes is 2.18 seconds and for controls 0.57 second. The period of contraction for rodless eyes averages 2.56 seconds while the controls average 3.73 seconds.

All pupils dilate to a maximum under the influence of atropin and contract to a minimum under the action of sulfide of eserine. It should be

TABLE 2

Normal diameter of pupil in albinos

RODLESS		NORMALS	
1	0.385	1	0.462
2	0.539	2	0.385
3	0.308	3	0.308
4	0.385	4	0.462
5	0.308	5	0.385
6	0.385		
Average = 0.385 mm.		Average = 0.396 mm.	

stated here that the two chinchilla rodless animals whose pupils dilated to 2.70 mm. have eyes larger than the other animals.

Normal contraction of the iris in albinos. In order to compare the results of chemically induced contraction in pigmented eyes with the state of contraction maintained normally by hyper-sensitive eyes, the diameters of the pupils of 6 albinos from a pure rodless family were recorded, with the pupillary measures for 5 animals of a normal-eyed family as controls.

That the contractions are all of the same order is readily noted by comparison of tables 1 and 2.

Table 2 shows the pupillary diameters for the two albino families mentioned above.

To test whether this was really a continuous state of contraction maintained normally by the albinos or whether it was a permanently small pupil only, drops of atropin were placed in the eyes of an example of each family. The iris in each eye dilated to the maximum.

DISCUSSION. We note that in every case the iris responded readily to photo-stimulation regardless of the functional capacity of the retina. This demonstrates the fact that in mammals the iris may function independent of vision.

The average latent period in the rodless eye is nearly four times that in the normal. Whether this difference of latent period represents the action of a reflex of the normal retina upon the iris or is due to some unknown structural defect of the iris accompanying the rodless condition in the house mouse, we are not in a position to say at present.

The actual reduction in the diameter of the pupil after contraction is 0.71 mm. in rodless eyes and 0.91 mm. in normal eyes. This is probably a significant difference. Here, too, we see an apparent lack of the precision found in the normal eye.

The diameter of the pupil is not a measure of the circular muscle of the iris but is related to it about as 1 is to 3.14. To learn the real rates of contraction we must compute the contraction of the actual muscle.

Linearally in the rodless mouse the iris muscle contracts from $(1.65 \times 3.14) = 5.18$ mm. to $(0.940 \times 3.14) = 2.95$ mm. or a total contraction of 2.13 mm. in 2.56 seconds or at the rate of 0.83 mm. per second.

Likewise calculating for the controls we get a contraction from $(1.53 \times 3.14) = 4.80$ mm. to $(0.602 \times 3.14) = 1.90$ mm. or 2.90 mm. in 3.73 seconds or at the rate of 0.77 mm. per second.

On the average the iris of the rodless eye contracts at a rate about the same as that in the normal eye.

The slight differences found by chemical stimulation of the iris are probably insignificant.

The lack of precision of the iris movements in the rodless mouse eye is very interesting from the standpoint of a study (unpublished) upon the responses to photic stimulation of the iris in an eye of a frog. This eye had been enucleated and replanted in its own socket. The iris of this eye responded readily to light a year after the operation but showed just such imperfections as compared to the normal eye in the same animal.

SUMMARY

As a result of this experimentation we are forced to conclude:

1. That the mouse iris under photo-stimulation dilates and contracts independent of the functional capacity of the retina, but, that
2. In the rodless mouse it requires a much longer time than in the normal for the iris to respond to light of the same intensity.
3. The iris of the rodless mouse contracts less than the normal under the same light stimulus.
4. The rates of contraction in the two types of eyes seem to be about the same.

5. The iris in both blind and seeing eyes responds equally to influences of atropin and sulfide of eserine.

6. The extreme contraction in pigmented eyes produced by sulfide of eserine is of the same order as that normally found in albino stocks no matter what the condition of the retina.

7. The extreme contraction of the albino iris of blind and seeing races is not necessarily permanent but may be readily overcome by the use of atropin.

8. The lack of precision in the action of the iris of rodless mice suggests a regulatory mechanism in the eyes of normal animals.

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VARIATIONS OF THE pH OF THE BLOOD AND THE RESPONSE OF THE VASCULAR SYSTEM TO ADRENALIN

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Attempting to further the hypothesis of a secretory innervation of the thyroid gland, Levy (1916) tested the response of the vascular system of the pithed cat to small doses of adrenalin following stimulation of the sympathetics. With the thyroids intact, either electrical stimulation of the cervical sympathetics, or a large dose of adrenalin (10 cc. of 1–100,000), or repeated small doses were thought to call forth thyroid secretion which reacted with the subsequent injections of adrenalin causing an increased vasoconstriction. This response reached a maximum in two or three hours, after which there was a gradual decline. No increase in response took place if the thyroids were removed before the experiment. The conclusion was drawn that stimulation of the sympathetics caused the thyroid to pour an excess of its secretion into the vascular system, which, after a latent period of an hour, rendered increased excitability to sympathetic structures acted upon by adrenalin in raising arterial blood pressure.

This evidence of a secretory mechanism for the thyroid was so striking that one of us (Burget) was led to repeat Levy's work. More than fifty experiments were carried out including a series under each of the following heads: 1, cervical sympathetic electrically stimulated; 2, given 5 cc. of 1–100,000 adrenalin; 3, thyroids removed; 4, thyroids removed and given 5 cc. of 1–100,000 adrenalin; 5, controls. In each series an increasing response to small injections of adrenalin was manifested. Composite curves of blood pressure before and at the height of adrenalin response are shown in figures 1 and 2. A sufficiently large number of animals in each of the five series would have unquestionably resulted in the curves coinciding. However, there remained a few experiments in each series that showed no increasing response. Blood pressure fell to such a low level in two to three hours that the animal was of no value.

It was obvious that the thyroid was not concerned in these results. Yet the questions remained as to what caused the increasing responses,

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and why some animals in each series failed to show any increased response. The data[2] were filed and the above questions kept in mind.

Meanwhile Lieb and Hyman (1922 a, b, c, d) published work showing exactly what we had found, namely, that the thyroid was not concerned in the increased responses of the vascular system of the pithed cat to small injections of adrenalin. They showed that this increased response was not due to an accumulation of adrenalin, an increase of blood bulk, a stimulation of the thyroid gland, or to an adrenal medulla or parathyroid insufficiency. The conclusion was drawn that there was involved a variable, not considered by Levy, the involuntary nervous system. This in their opinion underwent sensitization to adrenalin when repeated small injections were made. Proof, however, that such was the case could not be said to be conclusive.

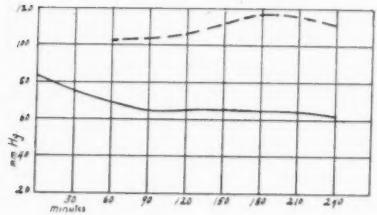

Fig. 1

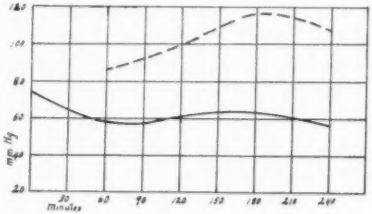

Fig. 2

Fig. 1. Curves of average blood pressure and height of response to 0.30 cc. of 1–100,000 adrenalin in 7 animals. Cervical sympathetics stimulated with induced current previous to administration of adrenalin. Adrenalin injected at 30-minute intervals. Solid line, blood pressure; broken line, curve of response.

Fig. 2. Curves of average blood pressure and height of response to adrenalin in 5 animals with thyroids removed previous to injections of adrenalin.

Having been led by some recent work in the literature to consider the possibility of the influence of the pH of the blood upon the response to adrenalin, a hitherto uncontrolled factor of possible importance presented itself. Accordingly we began to carry out further experiments on the pithed cat taking into consideration the pH of the blood at the time of adrenalin response.

The animals were prepared in the usual manner described by Elliott (1912), Levy (1916) and Lieb and Hyman (1922-a). The period of anesthesia was made as short as possible; pithing of brain and cord was carried out as soon as a tracheal cannula could be inserted following complete anesthesia. The cord was pithed to the lumbar region. If pithing was

[2] Presented before the Univ. of Oregon Med. Research Club, Portland, Oregon, October, 1920.

not well carried out to this region, the results were likely to be complicated by movements of the animal. Artificial respiration was begun at once. Care was taken to make it ample and over-distention of the lungs was avoided for reasons to be pointed out later. The temperature of the animal was kept more nearly constant by covering and applying the heat from an electric light globe. One carotid artery was used to register blood pressure while a cannula was placed in the other for collection of samples of blood. A cannula connected with a burette containing normal salt solution was placed in a femoral vein. For adrenalin injection a graduated 1 cc. pipette was used. On one end a hypodermic needle was attached and on the other a 5 cc. syringe. The adrenalin solution could thus be drawn into the pipette and injected with a considerable degree of accuracy into the rubber tubing connecting the vein cannula with the burette containing normal salt solution. The amount injected was always 0.30 cc. of a 1–100,000 solution prepared by diluting adrenalin (Parke, Davis & Co.) to 100,000 with 0.9 per cent NaCl before each experiment. Injections were rarely repeated under 10 minutes and usually a much longer period was allowed to elapse.

Immediately following the adrenalin injection a 1.5 cc. to 2.0 cc. sample of blood was drawn into a centrifuge tube in which one drop of saturated potassium oxalate solution had been evaporated and 2 cc. of paraffin oil added. The oxalate was distributed through the sample by gentle rolling after which the tube was placed in the centrifuge to bring down the cells. A pH determination was then made on the plasma by the colorimetric method at body temperature as described by Hastings and Sendroy (1924).

The Hastings-Sendroy adaptation of the bicolor standard principle to plasma pH determinations, has yielded very satisfactory results in this work. In the range of hydrogen-ion concentration between pH 7.15 and pH 7.50 the error in reading is less than 0.03 on the pH scale, and it seems that in most instances the readings are accurate, at least on a comparative basis in a series of samples, to 0.01 on the scale. Outside the range pH 7.15–7.50 the errors in color matching are much larger and no special reliance is to be placed on differences of less than 0.05 of pH.

Any great amount of hemolysis, with a consequent presence of hemoglobin in the plasma, makes an accurate color matching impossible. Hence the method is not useful with such samples of blood. Fortunately in these experiments there was almost no hemolysis in any but one or two samples of blood which were taken after traumatic injury to the lungs by extreme over-distention. Those few readings were discarded.

The injection of sodium carbonate was reported by Collip (1921) to cause a slight increase in the response of the vascular system to adrenalin while sodium acid phosphate caused it to decrease. Lieb and Hyman

(1922c) using the same substances obtained slight alterations in the response in the reverse directions. Given in what may be considered physiological doses these substances do not alter the pH of the blood greatly. On the other hand, we found alteration in the ventilation of the animal a most effective means for our purpose. Allowing the CO_2 to pile up causes a marked fall in the pH (Scott, 1917) while washing out the CO_2 by increased ventilation causes the pH to rise. This means was used in the following experiments for varying the H-ion concentration of the blood. When reducing the ventilation care was taken not to bring on asphyxia to the point of causing dilatation of the heart beyond recovery, or to introduce other factors. Very gradual accumulation of CO_2 was most satisfactory because it introduced no other variables. Figure 3 illustrates the point. When the pH had fallen sufficiently to cause a much reduced response to adrenalin the ventilation was increased to wash out the accumulated CO_2 after which the response increased. The animals responded remarkably well to increased ventilation, showing a rapidly rising pH of the blood, except in a few instances where there was an uncompensated acidosis. In these animals the blood could be made brightly arterial in appearance yet the pH remained low. This happened when pithing was incomplete and movements took place (fig. 4).

Increasing the blast of air supplied to the lungs of an animal to such an extent as to overdistend the lungs, will, as has been known from the time of Quincke and Pfeiffer (1871), increase the resistance to the flow of blood in the lung capillaries. Very great intra-tracheal pressure will completely occlude the lung capillaries, and even moderate pressures embarrass the action of the heart considerably. It should be noted that with most of the poorer methods of artificial ventilation there is a great tendency to use too great insufflation pressures. Strong blasts of air at short intervals are entirely unsatisfactory.

Fig. 3. Illustrating effect of gradual accumulation of CO_2 upon the pH of the blood and the response of the vascular system to intravenous injection of adrenalin. (In all experiments the amount of adrenalin per injection was 0.30 cc. of 1 to 100,000.) The ventilation in the beginning was ample for the circulatory conditions at the time as responses at 9:50, 10:02 and 10:12 indicate. At 10:35 the respiration was reduced slightly. From 10:38 until 11:45 the pH of the blood and the responses to adrenalin decreased. At 11:46 ventilation was increased and the pH rose from 6.95 at 11:45 to 7.30 at 11:55, reaching 7.33 at 12:00 while the response increased accordingly. Two and one-half hours later the blood pressure had fallen and the blood showed a lower pH.

Fig. 4. Uncompensated acidosis. The pH of the blood was 6.90 at the beginning of injections and 30 minutes later was found to be 7.00. Throughout the next two hours the blood pressure fell and the pH remained at 6.90 although the blood was arterial in appearance.

Fig. 5. Inadequate ventilation and failure to obtain any increased responses. The pH fell in three hours from 7.27 to 6.90 and the blood became quite dark in color.

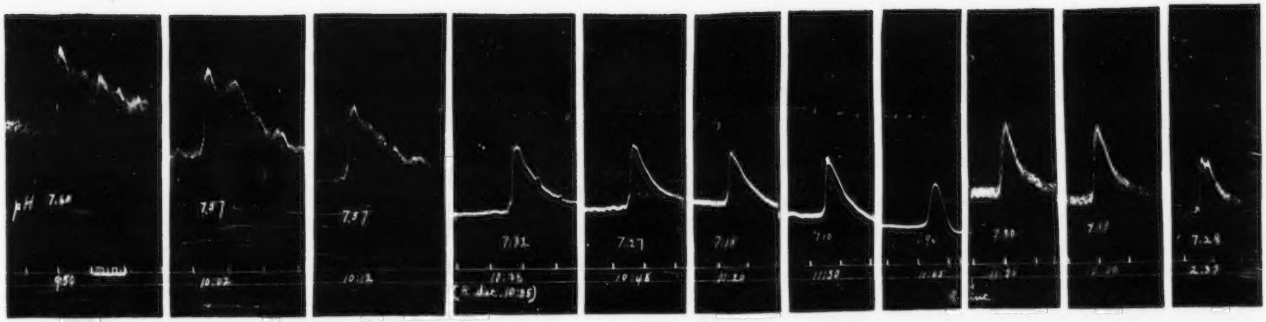

Fig. 3.

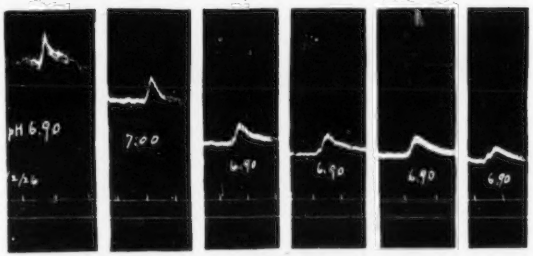

Fig. 4.

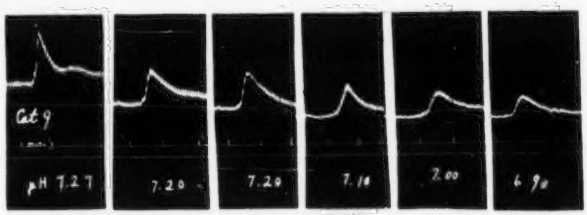

Fig. 5.

Continued inadequate ventilation, that is, inadequate from the beginning, brings about a gradually falling pH and no increased response (fig. 5).

Illustrations of ventilation, adequate in the beginning with high heart rate and rapid circulation, efficient in driving off excess CO_2 acquired in preparing the animal for artificial respiration and remaining unchanged throughout the experiment, are shown in figures 6 and 7. These experiments represent the typical result. Due to effects of the ether in producing increased acidity of the blood, and to accumulation of CO_2 incurred in preparing the animal, the early responses are relatively low. As these effects are overcome the response increases to a maximum during the third hour. The blood pressure now begins to fall and the circulation rate to decrease. These bring about an increasingly inadequate aeration of the blood. The pH of the blood falls and the response decreases accordingly.

In figure 8 is seen an example of extreme asphyxia of short duration. The blood pressure in this animal fell to a lower level than is shown, yet in 60 minutes of good ventilation, during which time no adrenalin was given, the pH rose to 7.36 and the response was correspondingly high.

Discussion. The investigations of Cushny (1908), Elliot (1912) and Schultz (1909) regarding the vascular response of adrenalin, led them to conclude that there was a remarkable constancy in this reaction. We can agree with this provided the H-ion concentration of the blood remains constant.

Lyon (1923a, 1923b) has stated that the higher the blood pressure the lower the response to adrenalin and that this reaction in the decerebrate cat obeys Weber's law. Our work shows no experimental evidence for such a statement.

Concerning the part the thyroid plays in causing an increasing response to repeated injections of adrenalin as stated by Levy (1916), our work confirms that of Lieb and Hyman (1922b) who found that the thyroid was not concerned in the reaction. Levy's work can hardly be considered as lending evidence to the theory of a secretory innervation of this organ. His results can be explained by assuming that he encountered an acidosis, either from lactic acid due to struggling after incomplete pithing or from accumulating CO_2 due to insufficient ventilation, in the thyroidectomized animals. Although it would seem to be a peculiar coincidence that such acidosis occurred only in those animals where the thyroid had been removed.

Lieb and Hyman (1922b) encountered the same phenomenon in some of their experiments. They state, "In the vascular response to repeated injections of similar doses of adrenalin, all known variables being carefully controlled, there is present in most, but not in all, animals a . . . . variable. This variable consists of a progressively increasing response." The increasing response they believe due to sensitization of the involuntary nervous system, probably at the myoneural junction. No explanation is

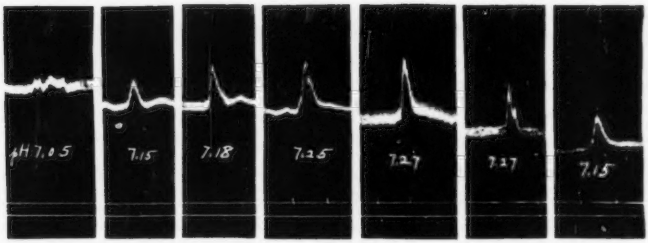

Fig. 6.

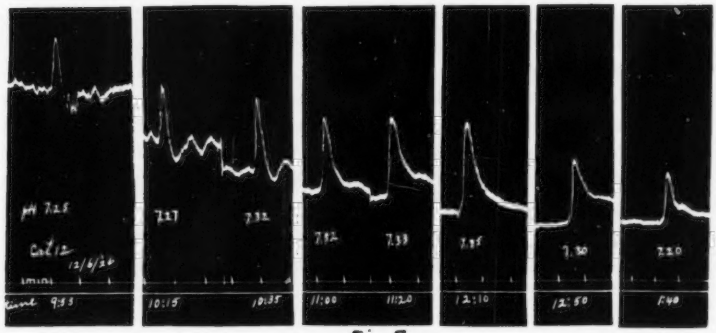

Fig. 7.

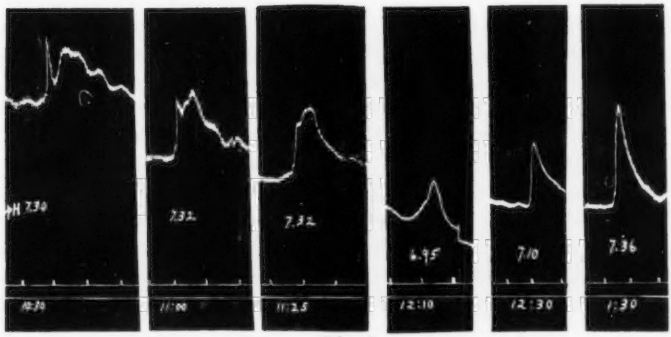

Fig. 8.

Fig. 6. Unchanged ventilation until the circulation began to fail. Acidosis, largely of CO_2 origin, is shown in the beginning. This was driven off and the pH reached 7.27 in 120 minutes with a maximal response; at the end of 180 minutes the pH was 7.15 and the response much less.

Fig. 7. Similar to figure 6 except that only slight acidosis was present in the beginning, pH 7.25. In 135 minutes the pH was 7.35 and the response maximal. At the end of 175 minutes it was 7.30 and at 225 minutes 7.20 with the response decreasing.

Fig. 8. Effect of producing extreme asphyxia of short duration. The ventilation was decreased at 12:05 and a response taken at 12:10 with a pH of 6.95. The blood pressure continued to fall until the ventilation was increased at 12:15. After 15 minutes of adequate aeration the pH was 7.10 and after 75 minutes it had risen to 7.36. At this time the response to adrenalin was very strong.

offered for their failures. They further state, without any explanation, that unless the ventilation is kept constant the effect of the adrenalin may vary. Such a statement is misleading. Varying the ventilation does cause the responses to vary. On the other hand it would be impossible to adjust the ventilation of an animal so well that the CO_2 would not either be gradually piled up or gradually washed out if the circulation remained constant. The situation is still further complicated by the fact that the circulation in these pithed animals does not remain constant. The blood pressure falls rapidly for the first 30 minutes after pithing and then holds its level for a time, eventually falling. The heart rate gradually slows down. Therefore it is unreasonable to assume that a constant rate of ventilation would keep either the carbonic acid or the pH of the blood at a constant level. Our work shows that the adrenalin reaction varies as the pH of the blood varies. Here then is the explanation for the variation in response when the artificial respiration and all other known variables, except the pH of the blood, are kept constant.

An interesting fact in connection with known properties of adrenalin is that its oxidation "is hastened by alkalies" (Mathews, 1916). Whether or not the increased response to adrenalin when the blood is more alkaline indicates that the physiological activity of adrenalin is bound up with its rate of oxidation one cannot say, but at least the coincidence of properties is suggestive. Whether this increased response with a high pH of the blood means that the adrenalin is more completely and rapidly oxidized and thus constitutes a stronger stimulus or that perhaps the sympathetic myoneural junction varies in irritability as the pH changes, is under investigation. It seems positive that the seat of action of adrenalin is not sensitized by repeated injections of the drug and that the hypothesis of Lieb and Hyman is rendered unnecessary.

Subcutaneous injections of adrenalin were tried by Lieb and Hyman (1922d) but they did not get increased responses "with any degree of regularity." Luckhardt and Koppanyi (1926) have shown that the response of the vascular system to subcutaneous injections of adrenalin depends upon a number of factors alluded to in their paper, massage being a *sine qua non*. This perhaps explains the failure of the above authors to get consistent responses.

The effects of asphyxia on the adrenalin-iris-light sensitivity reaction as elicited in the enucleated eye of the toad were studied by McCarrison (1924). He found that the action of adrenalin was disfavored during the later stages of asphyxia and concluded that this effect was due to the gradually increasing content of CO_2 in the blood. Alpern (1924) perfused the isolated rabbit's ear with adrenalin in Locke-Ringer solution at different H-ion concentrations. He found this solution effective at pH 6.6 to

6.8, not effective at pH 5.2 to 5.6, and more than usually effective at pH 7.8 to 8.1.

Snyder and Andrus (1919) make the following statement: "The effect of epinephrin upon the terrapin heart is in part a function of the H-ion concentration of the perfusate." Later Snyder and Campbell (1920) in studying the vascular reaction to adrenalin in perfusates of various H-ion concentrations found that when perfusing with a solution of pH 7.0 a dilatation followed when a very weak concentration of adrenalin was added to the perfusate. If the pH of the solution was increased to 7.8 a constriction followed and if adrenalin was added in like concentration a still further vasoconstriction was produced. This would indicate an increased efficiency of adrenalin to cause vasoconstriction as the pH of the perfusing fluid rises.

Using a lower pH index, Salant and Johnston (1924) found in the perfused frog heart that a concentration of adrenalin of 1:80,000,000 in Ringer's solution caused stimulation when the pH value was 7.60 to 7.90. Adrenalin became less effective as the hydrogen-ion concentration was increased and in solutions having a pH value of 6.5 to 6.70 the amount of stimulation was small and transitory.

Lutz and Wyman (1925) studied the response of the vascular system of the pithed cat to repeated injections of adrenalin. They found, "The onset of augmentation was correlated with an increase of the CO_2 capacity of the blood which continued after the augmentation had begun to disappear." Acids prevented the augmentation and alkalies caused an increased response. They interpreted the augmentation as a temporary improvement in the animal tissues followed by a moribund condition. This seems inadmissible in view of our findings.

That ether in considerable amounts depresses the response of the vascular system to adrenalin was shown by Wyman and Lutz (1925). This is as we would expect in view of the increased acidity of the blood in ether anesthesia (Leake, Leake and Koehler, 1923).

The importance of hydroxyl-ions in determining the response of the vascular system of the frog to adrenalin was pointed out by Schmidt (1921). Hemingway (1926) also found that in the perfused hind limbs of a cat there is an increasing reaction to successive changes in the hydrogen-ion concentration towards the alkaline side and that an exaggerated response to adrenalin accompanies the reaction to alkalies.

All previous studies upon the relationship of the hydrogen-ion concentration to adrenalin response have been made under rather abnormal conditions as regards the perfusion medium. Evidence that in the animal, intact except for the central nervous system, the hydrogen-ion concentration of the blood does actually regulate the response to intravenously

introduced adrenalin, is essential to the acceptance of the reality of the relationship. The data presented in this paper, we believe, furnish the evidence for this conclusion.

CONCLUSIONS

1. In the pithed cat, when the artificial respiration is constant, small intravenous injections of adrenalin act with progressively increasing effectiveness provided the pH of the blood is rising.

2. The secretion of the thyroid plays no part in this reaction.

3. Such increasing responses to adrenalin are not due to the drug sensitizing the sympathetic nervous system.

4. The adrenalin response of the vascular system of the pithed cat may be made to vary at any time by varying the pH of the blood. From pH 6.9 to 8.0 the response progressively increases.

5. The most effective means of varying the pH within this range is by varying the amount of air carried to and from the lungs.

6. Acidosis caused by acids other than carbonic is not overcome by increased aeration nor do animals showing such acidosis give increased responses to repeated injections of adrenalin.

7. The increased response seems to be due either to an increased irritability of the sympathetic nervous system because of an increase in the pH of the blood, or to the possibility that as the pH of the blood rises adrenalin is oxidized more rapidly and completely and thus constitutes a stronger stimulus at its seat of action, the irritability of the myoneural junction remaining constant.

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MICRO-INJECTION STUDIES OF CAPILLARY PERMEABILITY

I. Factors in the Production of Capillary Stasis

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From a study of the mechanism of lymph formation Starling (1896) concluded that since the capillary endothelium is normally impermeable to the proteins of the blood the movement of water through the capillary wall depends primarily upon the balance between capillary blood pressure and the osmotic pressure of the plasma colloids. An excess of capillary pressure would therefore cause water to pass toward the tissues, while a converse relation would lead to the movement of fluid into the blood. It is particularly emphasized that the vitality of the capillary wall is of paramount importance since injury increases its permeability to protein, with a corresponding reduction of the effective colloid osmotic pressure. According to this view, therefore, the direction and the amount of movement of water through the normal capillary wall is determined primarily by the level of capillary pressure in association with osmotic factors.

Krogh and Harrop (1921), however, observed that simultaneously with the capillary dilatation produced by urethane in 5 and in 25 per cent solutions the permeability of the endothelium increases. This is indicated microscopically by the development of "stasis," by which they refer to a concentration of the corpuscles produced by the rapid loss of plasma through a capillary wall whose permeability has been markedly increased. The viscosity of the concentrated blood causes flow first to become sluggish, and finally to cease entirely, as the venous capillaries become completely filled with solid cylinders of tightly packed corpuscles.

The term "stasis" as used by Elliott (1921), Lennartz (1921) and Stegemann (1924) merely designates a cessation of flow without reference either to escape of fluid, or to change in the ratio of corpuscles to plasma. In experiments dealing with capillary permeability, however, Krogh's use of the word has been adopted by Herzog (1925) and by Florey (1926) although in each case the important feature is the concentration of the red cells, to which stoppage of flow is incidental. To avoid confusion, however, it is in this latter sense that the word stasis is to be used in this paper.

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Krogh concludes (1922) that "no dilatation of capillaries involving mechanical stretching of the endothelium can take place without being accompanied by an increase in the permeability,—an increase which runs on the whole parallel to the degree of dilatation and which allows all the normal plasma colloids to filter off rapidly when the capillaries are strongly dilated." Comparison of the low pressures in the venous capillaries of the skin (Carrier and Rehberg, 1923) with the osmotic pressure of the plasma colloids, as determined in collodion sacs of graded permeability, led to the further conclusion that there is in almost all tissues an excess of the effective osmotic pressure over capillary blood pressure. Krogh therefore expresses the belief that capillary permeability depends primarily upon the degree of dilatation, while capillary pressure is of minor significance.

1. Factors to be considered in the production of stasis. The increase of permeability observed in urethane stasis is ascribed by Krogh and Harrop (1921) to pores having a diameter between five and two hundred millimicra which appear when the capillary wall is sufficiently stretched. Capillary pressure though not measured was believed to have no influence since stasis developed quite as easily after urethane, which leaves the arterioles relatively constricted, as after mechanical stimulation, in which case the marked arteriolar dilatation increases capillary pressure and flow. The possibility that urethane stasis might be related to the toxicity of the narcotic, or to the osmotic properties of the markedly hypertonic solutions used, is not considered.

There is evidence in the literature, however, that an increased passage of fluid through the capillary wall may be due to any one of several important factors of which the most obvious are: 1, increase of capillary pressure; 2, injury to the endothelium; 3, an osmotic withdrawal by hypertonic solutions, and 4, increased rate of blood flow with a rise of capillary pressure. In view of the evidence already accumulated in favor of Starling's view, it would seem that these more important possibilites should be directly eliminated before ascribing the increased permeability of any dilatation to an inherent change in the character of the capillary wall.

a. Capillary pressure. The low pressures found by Carrier and Rehberg (1923) in the venous capillaries of the skin tend to minimize the importance of capillary pressure as a factor in fluid interchange. However, the vessels penetrated were on the venous side of the capillary network. Kylin (1926) observes that with the use of pipettes having a diameter of 20 micra flow is blocked and the pressure actually measured is that of the vessel into which the penetrated venous capillary empties. Moreover, measurements made in but one portion of the peripheral circulation would not show the presence of a pressure gradient if one existed.

A series of direct measurements has been made by a micro-injection method at various points distributed through the peripheral circulation

of the frog's mesentery (Landis, 1926). Special precautions were taken to avoid any modification of flow in the observed capillary. By these determinations the presence of a gradient of pressure fall in the capillary bed has been established. In the arteriolar capillary the average pressure was 14.5 cm. water, and in the venous capillary 10.0 cm. White (1924) found the colloids of frog's plasma exerted an osmotic pressure ranging between 10 and 12 cm. of water. Hence the average capillary pressure in the arteriolar end is above, and in the venous end below, the colloid osmotic pressure. This indicates a balance between capillary pressure and the osmotic pressure of the plasma colloids as was originally suggested by Starling.

Moreover, direct measurements also indicated that probably in dilated capillaries and certainly in capillaries with high rates of flow there is a higher pressure than in constricted vessels. The independent variation of arterial and capillary pressures which is well established (Kylin, 1926) makes it impossible to infer the level of capillary pressure from measurements of systemic pressure, as is done by Tani (1924).

There is evidence also that a certain level of peripheral pressure is necessary for the development of edema. Tainter and Hanzlik (1924) found that following the intravenous administration of paraphenylenediamine, edema is prevented by lowering the peripheral pressure. This may be accomplished either by lowering systemic pressure with shock-producing doses of the drug, or by the administration of adrenalin, which though raising arterial pressure, lowers the capillary or filtration pressure. Hirschfelder (1924) found that the inflammatory edema of the conjunctiva following application of mustard oil could be prevented by peripheral vaso-constrictor agents such as cocaine, or adrenalin, which act by reducing capillary pressure. The importance of a sufficient filtration pressure is also indicated by the action of sodium nitrite. This vaso-dilator substance increases the inflammatory edema when locally applied; but when introduced by vein, though the same degree of dilatation is produced locally, no edema is observed because of the lowered systemic pressure. Hence the important element appears to be not dilatation but the increased pressure which accompanies it.

b. Injury to the endothelium. According to Starling any substance which by its toxicity partially or completely destroys the impermeability of the endothelium for plasma colloids would cause water to pass at an abnormally low capillary pressure. The gradual development of pulmonary edema in the heart-lung preparation is believed by Lambert and Gremels (1926) to be due to the injury of the pulmonary capillaries by toxic substances forming slowly in defibrinated blood. This damage could be histologically demonstrated. In the formation of urticarial wheals Lewis (1924) and Lewis and Grant (1924) have concluded that the

movement of fluid is independent of any increased permeability of vessel walls which might be due to distention, but is chiefly due to a heightened permeability caused by the liberation of a histamine-like substance after tissue injury.

The edema following paraphenylenediamine injection is believed by Tainter and Hanzlik (1924) to owe its gelatinous character to the abnormal permeability of the damaged endothelium for plasma proteins including fibrinogen. Hirschfelder (1924) also demonstrated by trypan blue that the inflammatory edema following application of mustard oil was due to a damaged endothelium and that epinephrin prevented the edema not by any direct protective action on the endothelium but by its reduction of peripheral pressure.

Jacobj (1923) describes the effects of applying formaldehyde to the frog web. There is observed first an increased flow, then a dilatation and concentration of the red cells with stasis. The description resembles that of Krogh for urethane stasis. The tendency of the corpusles to adhere to the capillary wall is given by Jacobj as evidence that both dilatation and increased permeability are the result of injury.

Herzog (1925) in repeating Krogh's experiments with 20 per cent urethane finds that in the frog's tongue capillaries thus dilated are not only more permeable but more sticky as well. Both effects are best observed in the areas rapidly passing into stasis, where, however, there is noted a definite tendency toward irreversibility. In addition the possibility of the toxic action of even dilute solutions of urethane is presented in the reduced or abolished reactivity of certain smooth muscles to pilocarpine and barium after their relaxation in 1 to 2 per cent urethane. Franklin (1925) found that a 1 per cent solution produces a relaxation from which recovery does not occur.

There is every indication that injury of the endothelium can increase permeability and there is some possibility as well that urethane itself is a toxic substance even in relatively small percentage.

c. Osmotic activity of the substance applied. Weed and McKibben (1919) described a diminution in brain size on the introduction of hypertonic salt solution into the blood stream. The results are explained as fundamentally osmotic effects, by which a hypertonic solution introduced into the blood stream withdraws water from the cranial cavity. If the hypertonic solution is applied outside the vascular system there is similarly a movement of water toward the higher osmotic pressure. Cushing and Foley (1920) found that concentrated salt solutions in the alimentary canal removed water from the intestinal capillaries, and then secondarily from the cranial cavity.

The isotonic strength of urethane is about 2.5 per cent. It has been noted by Krogh (1922) that a 1 per cent solution applied to the tongue

causes only increased rate of flow, and that 5 and 25 per cent both cause stasis. Since the latter solutions are markedly hypertonic and are the only ones effective in causing loss of fluid through the capillary wall, the possibility of an osmotic withdrawal should be ruled out before the concentration of the blood is ascribed to dilatation only.

d. Rate of blood flow in the observed capillary. In general, capillary pressure rises by amounts easily measurable by the direct micro-injection method as the rate of blood flow in the capillary increases. In abnormally permeable vessels, moreover, if active flow be maintained a minor loss of fluid is imperceptible. It becomes increasingly noticeable if the flow is slower and the same volume of blood is exposed to the concentrating factors for a longer period. This occurs as flow is made more sluggish in urethane stasis.

Lewis (1924) indicates that the formation of urticarial wheals is largely dependent upon the rapidity of blood flow in the stimulated area and apparently not related to mere distention.

It is the purpose of the experiments to be described to consider each of these factors in turn especially with relation to urethane stasis, upon which a certain amount of emphasis is placed in Krogh's hypothesis. The advantages of the micro-injection method lie in the possibility of observing and manipulating the single capillary while at the same time the pressure in the vessel is measured directly. The results indicate that certain of these factors play a great part in the production of stasis by urethane thus reducing the probable importance of dilatation and stretching as the sole cause of increased permeability.

2. Material and methods. The observations have been made upon the mesenteric capillaries of two species of frog (Rana pipiens and R. catesbiana). They are well adapted for observation, measurement of pressure, and the application of substances to their exterior. The use of a thin membrane lessens the possibility of dilution as penetration occurs.

The general method has already been described (Landis, 1926). The capillaries were viewed through a 16 mm. objective and a Zeiss photomicrographic ocular. Capillary pressure was measured through micropipettes having a diameter of 4–8 micra at the tip. The other end was connected to a column of water for the purpose of balancing the capillary pressure. A few minor improvements added to the ease and accuracy of making the determinations. For the sake of precision and firmness the frog board has been mounted on a mechanical stage. One of the major difficulties in the technique has been the ease with which the tips of the micro-pipettes are clogged by fine particles which are found, even after filtration, in the fluids to be injected. It has been advantageous to put the solutions for micro-injection into glass tubes of 2 mm. bore. After both ends are sealed in a flame the tubes are centrifuged at high speed for 3–5

minutes. The lower portion of each vessel with some of its contained fluid is broken off. This removes the detritus which clogs the micropipette and leaves the remaining solution clearer than any ordinary filtrate.

3. Observations. *a. Relation of high capillary pressure to passage of fluid.* Capillary pressures as high as 24 cm. of water have been observed in the normal mesentery. But the exceedingly rapid flow under these circumstances prevents the production of any observable concentration of the corpuscles. The passage of fluid at these high, virtually arteriolar, levels can be made obvious, however, by raising pressure and simultaneously stopping flow. This can be done by very gentle pressure of the microscopic glass rod upon the venous end of a capillary arising directly from a large arteriole in which flow is rapid.

This raises the pressure in the entire length of capillary to the arteriolar level. Under these conditions without any change in capillary diameter the corpuscles slowly move together as fluid is filtered through the wall. Even full arteriolar pressure, however, does not produce stasis if the capillary wall is normal, some plasma remaining between the corpuscles even after 15 minutes of pressure well above 25 cm. of water. The closely packed cylinder of corpuscles characteristic of stasis is not formed, presumably because with concentration the osmotic pressure of the colloids is increased until the higher filtration pressure is balanced. Immediately on the removal of the obstruction to flow the cells are washed out of the capillary into the venule and normal circulation is resumed.

The passage of fluid here cannot be due to lack of oxygen since the exposed mesentery is well aerated, nor to dilatation since the diameter remained constant. The concentration depends then upon a high filtration pressure. It can be stopped at any stage simply by the compression of the arteriole or of the arteriolar end of the capillary and is immediately resumed when the obstruction is removed from the arteriole and pressure again rises in the impeded capillary. When the arteriolar pressure is low, i.e., below 18 cm. water, there is little or no visible concentration. Even with the high pressures of 28–30 cm. water some plasma is retained due to the osmotic properties of the colloids to which the capillary wall, being normal, is impermeable.

b. The production of stasis by injury of the capillary wall. In certain preliminary experiments dye solutions were injected through a micropipette inserted into the capillary lumen. It was observed that the dye at once passed through the portion of capillary wall that had been damaged in the course of the insertion of the tip of the pipette. It required some minutes on the other hand for the dye to appear outside the normal endothelium. It was concluded, after ruling out the possibility of leakage that mechanical pressure increased the permeability of the capillary wall by an injury effect.

Fig. 1

Fig. 2

Fig. 3

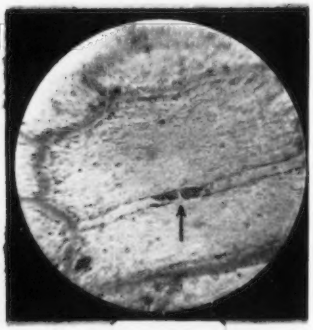

Fig. 4

Fig. 1. Photomicrograph (× 80) showing the perfusion of a normal capillary with toluidine blue in Ringer's solution. After 4 minutes' flow at 10 cm. pressure there is no visible passage except to the left where the wall has been injured by the insertion of the micropipette.

Fig. 2. The same capillary is shown thirty seconds after the wall was locally compressed with the blunt glass rod. The point of injury is indicated by the arrow. The passage of dye in this area indicates the increased permeability resulting from injury.

Fig. 3. Photomicrograph (× 80) showing the increased permeability following mechanical injury. In contrast to the dye India ink is retained by the capillary wall as the plasma escapes, indicating that the increased permeability is not due to a tear of the endothelium. Concentration of the ink, and hence filtration of plasma, was greatest on the arteriolar side, to the left. Arrow denotes the injured area.

Fig. 4. Photomicrograph (× 80) showing local concentration of cells and stasis produced by mechanical injury. Arteriole is to the left. Arrow indicates the injured area.

This increase of permeability to a Ringer's solution containing dissolved dye is indicated in figures 1 and 2. The micro-pipette was introduced at the left of the upper capillary and dye perfused, the pressure being 10 cm. water. At the end of four minutes the first photomicrograph was taken (fig. 1) showing no perceptible passage at any point except on the left where the pipette has injured the wall. At this time the capillary was compressed by the glass rod and a second photograph taken 30 seconds later shows a marked passage of the dye solution through the injured area, while there is still no passage through the normal capillary wall.

The fluid does not spurt from a single point as would be the case if the endothelium had been ruptured but filters outward uniformly along the whole damaged section. To demonstrate that compression does not produce a tear in the capillary wall, India ink was introduced into the ventricle of a frog until the plasma became a homogeneous grey in color. If a capillary is then injured by compression as before, even after severe pressure the India ink does not appear outside the wall, but is concentrated along with the corpuscles as plasma escapes (fig. 3). In fact the India ink outlines the damaged area very clearly by adhering to the abnormally permeable portion of the endothelium. Finally corpuscles and black pigment are commingled in the plasma-free collection characteristic of stasis.

These results indicate that in mechanical injury there is a marked increase in permeability which, while permitting the immediate passage of dye in Ringer's solution, is not sufficient to allow the particles of India ink to pass. This is therefore an increased permeability without change in capillary diameter. Flow has been stopped by the introduction of the micro-pipette and pressure throughout the length of the capillary is that of the arteriole or venule to which it is connected. Differences in filtration pressure cannot explain the results since two areas of a single capillary are being compared. In this instance then injury of the endothelium seems to be the sole factor in the change of permeability.

According to Krogh (1922) India ink is similarly filtered off in the concentration and stasis produced by urethane. Herzog (1925) describes the aggregation of India ink particles on the inner surface of the endothelium wherever stasis is produced whether by mechanical pinching, application of urethane, injection of arsenical compounds or by hot water.

Typical concentration of the red cells and stasis can also be produced by exerting firm pressure on a normal capillary by means of the blunt rod. With the continued loss of plasma through the injured area the corpuscles move slowly toward the damaged zone from both extremities of the capillary. The slowness of movement leads to plasma skimming at the two extremities of the vessel since movement of fluid is not rapid enough to cause corpuscles to leave the axial stream of either arteriole or venule. The mechanism of this plasma skimming has been described previously (Landis,

1926). Finally there results the condition photographed in figure 4. The cells which previously were equally distributed throughout the entire length of the capillary are now packed in one mass, the remainder of the vessel being filled with plasma alone.

When a large venous capillary is thus compressed concentration and stasis occur in the same manner. But usually the central cells in the mass are slowly pushed forward by the pressure on the arterial side. Finally they are forced completely through and circulation is resumed through a hollow cylinder of erythrocytes which are closely adherent to the damaged endothelium. This stickiness of the injured wall is very characteristically seen after the capillary is injured either chemically or mechanically.

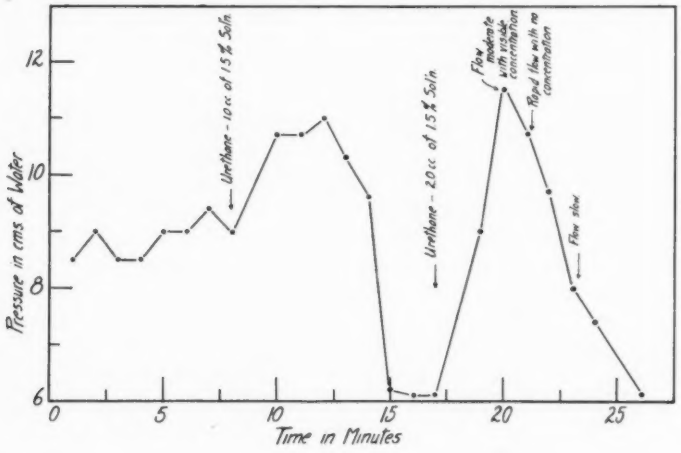

Fig. 5. Chart showing rise in capillary pressure following two applications of 1.5 per cent urethane in Ringer's solution to the mesentery.

Stasis of this sort while independent of change in capillary diameter is to some extent dependent on capillary pressure. The rapidity with which concentration is effected is modified by the filtration pressure. The movement of the corpuscles toward the abnormally permeable area is rhythmic in character, the chief passage of fluid occurring during systole when pressure is high. The collection of cells or of India ink occurs much more rapidly on the arteriolar side than on the venous side (fig. 3) and indeed concentration may be entirely absent on the side of lower capillary pressure. In capillary nets with higher rates of flow, accompanied by increased capillary pressure, concentration and stasis occur more rapidly. If the pressure is generally low even severe injury is often unproductive of

stasis. Moreover the concentration may be immediately stopped at any stage if the arteriole is blocked and pressure thereby allowed to fall.

It seems clear that in mechanical injury there is an increased permeability of the capillary wall, and the stasis thus produced is independent of diameter change, but is modified by the level of capillary pressure. In addition, stasis has been produced by a series of substances which act by killing the tissues. Water of sufficiently high temperature, acids and alkalies, formaldehyde, ether, chloroform and alcohol in sufficient strength all increase the permeability to this extreme extent. In each case the first notable change is the tendency of the erythrocytes to stick fast to the

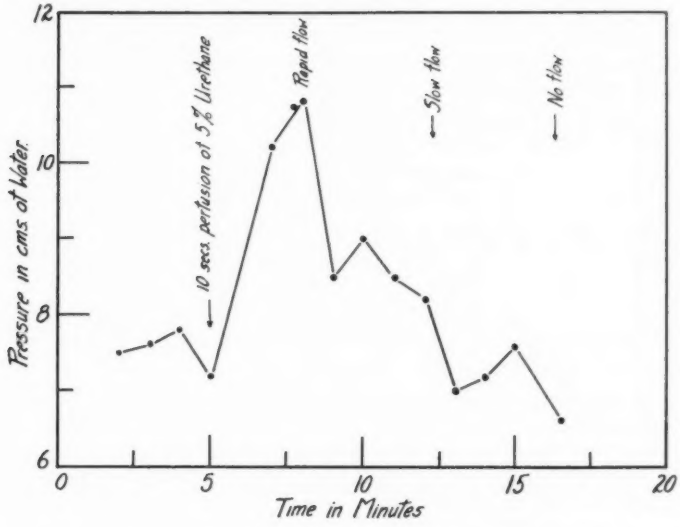

Fig. 6. Chart showing rise in capillary pressure following injection of 1.5 per cent urethane in Ringer's solution into a capillary network for 10 seconds.

endothelium, which is very shortly followed by the development of typical stasis.

c. The stasis produced by urethane. (1) The effect of urethane on capillary pressure. Direct measurement indicates that urethane in 1 per cent strength, which produces only a moderate dilatation and increased flow without stasis, increases capillary pressure by 2 to 6 cm. of water simultaneously with the increase in flow. Figure 5 shows the rise of capillary pressure obtained by the application of 1.5 per cent urethane in Ringer's solution, which sometimes produces a slight concentration when the pressure rises above 11 cm. If the urethane be introduced by micro-pipette into a

capillary network the same effects are noted. Figure 6 indicates a rise of capillary pressure of almost 4 cm. after ten seconds' local perfusion back to the arteriole with 1.5 per cent urethane. Direct measurement therefore shows rise in pressure coincidently with the slight initial dilatation and increased flow, which would tend to cause a greater passage of fluid from the blood stream.

When higher percentages of urethane are used, simultaneously with the sluggish flow and the beginning of concentration there is a marked and sudden rise of pressure to the level characteristic of arteriolar pressure, i.e., in excess of 25 cm. This marked rise of pressure (fig. 7) is due to the

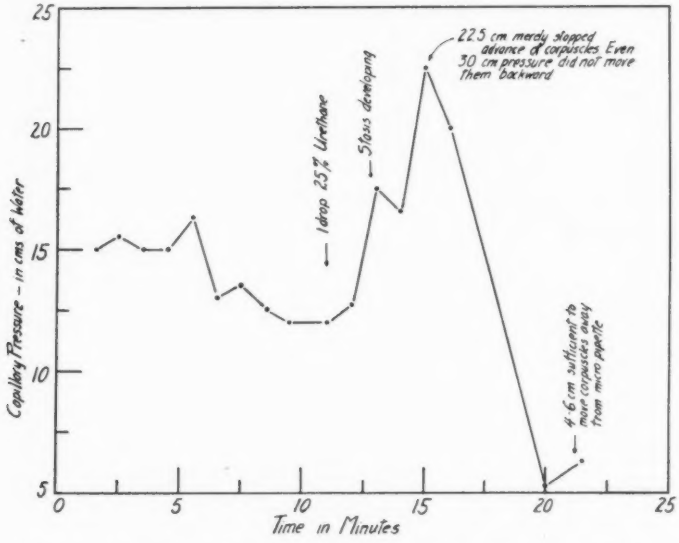

Fig. 7. Chart indicating the changes in capillary pressure occurring during the production of stasis by 25 per cent urethane.

blockage of the venous capillaries. If, as the pressure is rising, the cells collected in the venous capillary are washed out by a strong current of Ringer's solution from the pipette the pressure falls, only to rise again as the leakage of plasma once more makes the blood more viscous and impedes venous outflow.

With the reduction and finally the total stoppage of venous outflow, capillary pressure in the affected region rises until it equals arteriolar pressure. Plasma-skimming then results and this somewhat reduces the number of cells entering the vessels passing into stasis. Nevertheless due to the higher pressure the passage of fluid is quite rapid, and those

cells entering the capillary are packed together forming a solid cylinder which gradually extends from the venous to the arteriolar extremity of the capillary network. As this occurs the pressure is high manifestly due to a blocked venous outflow, producing the peak in capillary pressure shown in figure 7 during early stasis.

A few minutes later, however, 4 to 6 cm. water pressure is commonly sufficient to cause the corpuscles to move away from the tip of the pipette. This striking fall of capillary pressure occurs after the progressive packing of cells has reached the ends of the finest arterioles. It occurs because the cells collected in the ends of the arterioles have interposed a block to the arterial pressure.

That a degree of capillary pressure apparently something above 10 cm. is required to produce stasis with urethane is indicated by the cessation of concentration when arteriolar flow is blocked. Stasis is difficult to obtain when the animal is partially exsanguinated, probably due to the very low peripheral pressures resulting from arteriolar constriction. In addition in the normal animal the movement of corpuscles here, as in mechanical injury, is pulsatile in character, more rapid during systole than during diastole, indicating that capillary pressure is a factor in determining the rate of filtration of plasma.

The increase of pressure, however, cannot be the chief factor in the production of urethane stasis, since it has already been shown that in a normal capillary a pressure equivalent to that in the arteriole is insufficient to cause stasis, even when flow is stopped. The initial rise of 2 to 6 cm. therefore while aiding passage of fluid cannot be the real cause of stasis. The excessive rise in pressure later is the result first of increased viscosity of the concentrated blood, and later of absolute block of venous outflow. This high pressure while aiding the concentration is not the cause of stasis but one of its effects.

(*2*) *Relation of stasis to dilatation.* If Krogh's hypothesis is correct stasis should only occur in vessels which dilate at the time the increased permeability becomes evident. In the frog mesentery as prepared for these experiments the capillaries are as a rule moderately dilated within a few minutes after the tissues are exposed. Nevertheless circulation proceeds normally for hours if the surface is kept carefully covered with Ringer's solution. On the application of urethane to these dilated capillaries, stasis has frequently been observed to occur without measurable increase in the diameter of the vessels affected.

Photomicrographs have been prepared showing a capillary network before application of urethane and afterward when in stasis. A typical comparison of the diameters of the same capillaries before and after stasis is given in table 1.

With the higher concentrations even constricted capillaries frequently

pass rapidly into stasis, without change in diameter. Thus the corpuscles were barely able to enter the 4 constricted capillaries shown in stasis in figure 8.

Stasis produced by concentrated urethane is usually irreversible but with 1.5 per cent urethane in Ringer's solution flow is sometimes resumed. An example of such reversible stasis is represented in figures 9 to 11. The normal flow preceding the experiment is shown in figure 9. The short capillary *a* was only partially occupied by moving corpuscles. Due to this partial plasma-skimming one endothelial wall is clearly visible. With the sluggish flow of early stasis the stream of corpuscles was widened until it occupied the entire lumen. The appearance was that of dilatation but the difference lay only in the degree to which the lumen was filled with cells. By actual measurement the diameter of this capillary remained the same, though 13 minutes later the vessel passed into complete stasis. The distance between the endothelial walls had not changed during this period of increased permeability. Figure 10 shows the vessel in stasis.

TABLE 1

CAPILLARY NUMBER	CAPILLARY DIAMETER BEFORE URETHANE AND WITH NORMAL CIRCULATION	CAPILLARY DIAMETER AFTER URETHANE AND WITH COMPLETE STASIS
	micra	*micra*
1	23	23
2	9	10
3	17	16
4	16	16
5	17	20

Furthermore after a short period this stasis resolved with resumption of flow, but there was no measurable change in capillary diameter as shown in figure 11.

Though circulation returned after a short period of stasis it is to be noted that a few corpuscles have adhered to the endothelium. This recalls the stickiness of the mechanically or chemically injured capillary wall and suggests the possibility that the increased permeability produced by urethane may be due to toxicity.

(*3*) *The toxicity of urethane.* The stasis of urethane is almost always irreversible. In addition strong solutions (20 to 25 per cent) cause the corpuscles within the capillaries and the melanophores in the mesentery to disintegrate and give up their pigment. Stickiness of the endothelium appears to a marked degree when 5 per cent urethane is injected into the lumen of a capillary, and as mentioned above even in a reversible stasis produced by 1.5 per cent urethane. All these points suggest an injury of the capillary wall by the narcotic.

To determine the degree of toxicity for living cells, Arbacia eggs were fertilized and placed for development into sea water to which had been added urethane. A 2 per cent solution of urethane in sea water killed

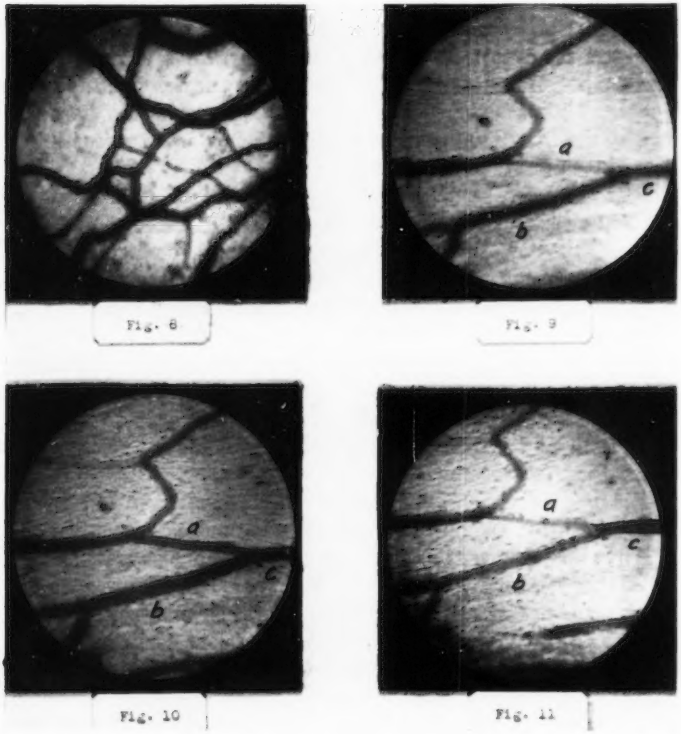

Fig. 8. Photomicrograph (× 80) to show concentration of corpuscles and stasis in constricted capillaries after urethane.

Fig. 9. Photomicrograph (× 80) showing circulation before the application of 1.5 per cent urethane in Ringer's solution to the mesentery.

Fig. 10. Photomicrograph of same field taken 13 minutes later showing total stasis in *a* without measurable change in diameter. The venous capillaries *b* and *c* are also in stasis, but in the remaining vessels circulation still persists.

Fig 11. Same field about 20 minutes later showing resumption of flow in *a* and *b* after urethane stasis without change in diameter. The corpuscles adherent to the walls of the capillary and venous capillary suggest injury of the endothelium.

all the eggs in the one-celled stage. Half of those in 1 per cent urethane failed to divide and none divided more than twice. In 0.5 per cent development was delayed but otherwise appeared fairly normal.

In addition the Arbacia eggs were exposed for increasing periods to solutions of urethane in sea water. After two rinsings they were returned to pure sea water and their development followed. An exposure of one minute to 25 per cent urethane in sea water sufficed to kill the eggs with a liberation of their pigment, much as the corpuscles give up hemoglobin in the presence of this strength. All the eggs were killed after a 5-minute exposure to 5 per cent urethane; 90 per cent were killed after two minutes, while with a one minute exposure the cleavages were delayed and markedly abnormal. With 1.5 per cent solution the early divisions were slightly delayed by the five minute exposure, while there was no perceptible effect produced by 1 minute in the solution.

Similar results were secured by observing motility of Paramecium caudatum in hay infusion to which urethane had been added. In 25 per cent and 15 per cent solutions death and cytoplasmic disintegration was immediate. Five per cent urethane immediately slowed their movement and killed within 5 to 7 minutes; while 1.5 per cent though markedly slowing movement after 5 minutes, did not kill in 20 minutes.

There is evidence therefore that urethane in the stasis-producing concentrations is a toxic substance and the increased permeability produced by this narcotic is believed to be due therefore primarily to injury of the capillary wall and not to dilatation. Where dilatation does occur it is secondary to the tissue damage.

(4) *The osmotic properties of urethane in the production of stasis.* The ineffectiveness of an isotonic solution and the marked concentration with the hypertonic 5 per cent and 25 per cent solutions suggest an osmotic withdrawal of fluid. It has been found impossible, however, to demonstrate any such action, apparently because of the rapid penetration of the capillary wall by the drug. There is not sufficient semi-permeability to set up more than a transient osmotic flow of water toward the hypertonic solution.

A 2.5 per cent solution of urethane in distilled water (the isotonic strength) produces the same effects upon corpuscles and the capillary wall as distilled water alone. If urethane produces concentration by osmotic activity it should be devoid of effect if introduced by micro-pipette into the capillary lumen. It was found, on the contrary, that injection of 1.5 per cent as well as the higher concentrations was accompanied by the production of stasis in the vessels through which the solution passed. The effect must be a direct injury, therefore, since osmotic withdrawal cannot act in such a case.

Moreover, all attempts to show directly the osmotic withdrawal of fluid have been unsuccessful. A capillary was closed by gentle compression at its two extremities, thus forming a sac of endothelium filled with plasma. The application of 5 per cent urethane did not produce a measurable

change in diameter and the cells were not noticeably concentrated. As soon, however, as the obstructions were removed the pressure caused rapid production of stasis. It is concluded therefore that osmotic factors play little part, that the permeability increase is due chiefly to direct injury.

Discussion. Krogh's theory of capillary permeability rests partly upon the assumption that the increased passage of water observed after the application of urethane to the peripheral circulation is due to the capillary dilatation produced. Consideration of the other possible factors has indicated that this is not the case. The two primary factors in urethane stasis appear to be injury of the endothelium accompanied by a high capillary pressure.

It is shown that abnormally high filtration pressures alone, though producing visible concentration do not produce stasis, probably since the normal wall is impermeable to the plasma colloids. The primary cause of the increased permeability after urethane is the toxic effect of the drug on the capillary wall. The rise of pressure is the result of the blockage of venous outflow, and while aiding the production of stasis it does not initiate the concentration of corpuscles. Lambert and Gremels (1926) similarly found that a rise of pulmonary arterial pressure accompanies the production of pulmonary edema in the heart-lung preparation. They conclude that the rise in pressure is the result of the increased viscosity of the concentrated blood and that injury is therefore the primary cause of the abnormal passage of fluid.

These experiments further indicate the importance of the determination of capillary pressures in experiments having to do with permeability of the capillary wall. It is impossible by any method to infer from arterial pressure determinations what may be the level of capillary pressure at any moment because of the complexity of the factors concerned in peripheral resistance. Frequently pressure is not measured, or it is thought sufficient to record arterial pressures. That the latter may be actually misleading rather than helpful is indicated in the effect of adrenalin which in preventing edema produces all the effects of a lowered capillary pressure, and yet increases the arterial pressure.

An increased passage of fluid has been observed in various forms of active hyperemia. Rogowicz (1885) found that stimulation of the lingual nerve increased the rate of passage of dye through the walls of the tongue capillaries. Cutting the sympathetic resulted in a more rapid staining of the ear on the affected side. Local heating of the skin, which produces peripheral dilatation and rapid flow was observed by Okuneff (1924) to increase the passage of trypan blue from the blood stream into the heated tissues. Anitschkov (1924) in reviewing the mechanism of the passage of colloidal dyes through the endothelium concludes that any active hyperemia increases the passage of dye.

The study of urethane stasis has indicated that there is no close correspondence between capillary diameter and the degree of permeability. Direct measurements by micro-injection have indicated a balance between capillary pressure and the osmotic pressure of the plasma colloids in the frog. It seems probable therefore that the explanation of the increased filtration of active hyperemia rests with the rise in capillary pressure which accompanies arteriolar and capillary dilatation, rather than the increased permeability supposed to exist in the stretched endothelium of the dilated capillary.

The consideration of the various possible factors in fluid movement will probably reduce the number of substances which are now believed to act by a direct modification of the normal endothelial permeability. Petersen, Levinson and Hughes (1923) found that epinephrin causes chiefly a diminution in the quantity and protein content of the lymph, and attribute its action to an "inhibition" of the endothelium. Injection of pituitrin, which resembles epinephrin in its effect on peripheral tonus has been shown by Bayley, Davis, Whitman and Scott (1925) to diminish the volume of lymph collected from the thoracic duct and to delay the exit of physiological salt solution from the blood stream. They attribute the result to direct action of pituitrin on the endothelium. Lee (1925) has found that histamine produces a marked fall in cerebrospinal pressure coincidentally with the lowering of arterial and venous pressure. Microscopic observation through a window in the skull provided evidence of dehydration of the brain substance. Though recognizing that the effects are complicated by circulatory factors, he postulates the functional difference of the cerebral capillaries and advocates the consideration of an active participation of the endothelial cells in explaining the phenomena of capillary permeability. Capillary pressure determinations in instances of this kind would be of the greatest interest.

Definite proof of the quantitative action of capillary pressure as distinct from systemic blood pressure on the passage of fluid through the endothelium is lacking. But there is sufficient indication of its importance as a factor in both normal and injured vessels to require that it be measured in any problem concerned with capillary permeability. The accumulation of such data will be of the greatest value in determining the true mechanism of the transfer of water and dissolved substances through the capillary wall.

SUMMARY

The possible factors in the production of concentration of corpuscles within the capillary are considered.

In the capillaries of frog mesentery a rise of capillary pressure to the

arteriolar level is accompanied by a slow concentration of corpuscles, but the filtration of fluid is not sufficient to cause stasis.

Typical concentration of cells and stasis, such as described for dilated capillaries by Krogh, can be produced locally by mechanical injury without change in diameter. The filtration of plasma is influenced by capillary pressure.

Direct measurement by a micro-injection method indicates that dilute urethane causes a rise in capillary pressure of 2 to 6 cm. of water, later followed by a further increase to above 25 cm. as stasis appears in the venous capillaries.

Increased permeability to urethane has been observed to occur in constricted and in dilated capillaries without change in diameter. In the strengths required to cause increase of endothelial permeability, urethane kills Paramecium caudatum and developing marine eggs.

In view of these findings it appears that the increased passage of fluid observed after application of urethane is not due to dilatation but to injury of the capillary wall, accompanied by an increase in capillary pressure.

These facts are opposed to Krogh's theory of capillary permeability, which is based largely on the view that the increased permeability after the application of urethane is due solely to dilatation.

It is a pleasure to express my gratitude to Prof. M. H. Jacobs who first suggested the application of the micro-injection technique to the problems of capillary permeability.

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THE AUGMENTED SALIVARY SECRETION

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Bradford (1) was the first to see a beneficial effect of a previous stimulation of the chorda tympani on the physiological activity of the salivary gland, induced by a subsequent stimulation of the chorda tympani or sympathetic. Langley (2) studied systematically the increased salivary secretion provoked by sympathetic stimulation after a previous stimulation of the parasympathetic nerve, and gave the name of "augmented secretion" to this phenomenon.

The ability of a nervous impulse to increase the response of the gland to a subsequent stimulus is not, however, limited to the parasympathetic nerve. This seems to be a universal property of the salivary secretory nerves. The augmented salivary secretion may be observed, not only in the case of stimulation of the sympathetic nerve after the chorda tympani, or Jacobson's nerve, but also in the case of any two successive nerve effects, i.e., in the case of stimulation of chorda after chorda (in the dog) (1); sympathetic (in cat) (3); chorda after sympathetic (in cat) (3); sympathetic after chorda (in dog) (1), (2); (in cat) (2), and sympathetic after sympathetic (in cat), (3). Therefore we may enlarge our conception of "augmented salivary secretion," and admit, with Goldenberg (3), that the augmented secretion may be obtained by successive stimulation of the same or of the two secretory nerves.

There are two opinions as the mode of production of augmented secretion. The original idea of Langley (2), supported by others (Maevsky (4), Goldenberg (3)), is that the augmented secretion is an expression of a raised excitability of the gland. The previous stimulation of one nerve produces an increased transitory irritability to the subsequent impulses reaching the glandular elements through another, or the same, secretory nerve. Another point of view, expressed by Mathews (5) and recently supported by Anrep (6), is that the augmented sympathetic secretion (Langley's case), results from the emptying from the gland of stagnant chorda saliva, due to contraction of some contractile elements of the gland through sympathetic nerve action. Mathews denies any secretory function of the sympathetic nerve. Anrep, on the other hand, acknowledges the existence of secretory fibres in the sympathetic. Both authors, how-

ever, look upon the augmented sympathetic secretion as a purely mechanical phenomenon. The evidence presented by both groups of investigators is quite strong and it seemed to us advisable in seeking a solution of the problem to attempt to approach it with new methods. This was done in the work here reported.

Massage of the gland. Some preliminary experiments with massage of the gland led us to the supposition that the phenomenon of augmented secretion is probably a complex one. It seemed to us that two factors may be causative: *a*, an activity of contractile elements of the gland under the influence of sympathetic nerve action; *b*, a true increased activity of the glandular elements resultant upon repeated stimulation of the nerves.

We present an experiment (expt. 1), which led us to this supposition, as an example from a series of analogous experiments.

In order to attain favourable conditions for the action of the sympathetic nerve in the dog we injected into the blood a solution of sodium carbonate, which according to Koupalov and Smirnitskaia (7) and Koupalov and Doushko (8), raises the irritability of the salivary glands and increases their blood supply. There was no blockage of the salivary ducts with viscid sympathetic saliva, which, in this experiment, seemed to be much thinner than usual. During the whole experiment, which lasted 3 hours, only the sympathetic nerve was stimulated. Notwithstanding this prolonged period of stimulation there was a marked sympathetic after sympathetic augmented secretion. The same phenomenon was seen by Goldenberg (3) in the cat, in which animal the sympathetic saliva is thinner than the chorda saliva.

Expt. 1. Dog. chloralose. Cannula inserted into the left submaxillary duct. Chorda tympani and sympathetic cut. To the cannula is connected a graduated glass tube, calibrated so that 10 divisions represent 0.075 of saliva. Secretion noted each 15 seconds. The numbers in *heavy type* show the period of sympathetic stimulation. The numbers in parentheses represent the amounts of saliva pressed out from the ducts by means of massage. Between 2:40 p.m. and 3:54 p.m., on four occasions, 10 cc. of 10 per cent sodium carbonate solution were injected into the blood. Coil at 7 cm. (the electric cell was weak).

a. 3:40 p.m.—*0*, 0, 0, 0, (2), (0). Total 2 div. (Secr. 0, Mass. 2).
3:42 p.m.—*0*, 0, 1, 3, 1, 1, 0, (6), (0). Total 12 div. (Secr. 6, Mass. 6).
3:45 p.m.—*0*, 0, 0, 2, 3, 1, 1, 1, 0, (6), (0). Total 14 div. (Secr. 8, Mass. 6).
No stimulation between 3:45 and 3:56 p.m.

b. 3:56 p.m.—*0*, 0, 0, 0, 0, (4), (0). Total 4 div. (Secr. 0, Mass. 4).
3:59 p.m.—*0*, 0, 0, 2, 1, 0, (5), (0). Total 8 div. (Secr. 3, Mass. 5).
4:02 p.m.—*0*, 0, 0, 2, 1, 1, 0, (4), (0). Total 8 div. (Secr. 4, Mass. 4).

As one can see, a subliminal stimulation of the sympathetic, ineffective during the first application of the current, provoked an augmented sympathetic secretion. The secretion began 45 to 60 seconds after the end of the stimulation and lasted 30 to 75 seconds, which makes any partici-

pation of the muscular elements of the gland in the production of the continued flow most improbable. The massage of the gland in each set of stimulations gave similar amounts of saliva. (The same phenomenon was observed in many other experiments.) This result shows that a definite part of the saliva secreted was retained and then pressed out, by massage, from certain parts of the salivary passages. Even if only a part of the stagnant saliva could be pressed out from the gland by massage, the difference between the amount obtained from the first massage (3:40 p.m.) and in subsequent applications, and the increase of secretion, when the massage gave equal amounts of saliva, speaks against the purely mechanical explanation of the phenomenon observed. (The difference between part *a* and part *b* of the experiment is that in the part *b* the gland was already in a certain state of excitation, and in part *a* the stimulation was begun after a long period of rest.)

This and analogous experiments led us to the above supposition concerning the dual mechanism of augmented secretion. We tried to verify and analyze this supposition by means of the following experiments. (We discuss neither here nor later the fundamental nature of the supposed "true augmented secretion." This must form a subject of a special investigation. But we should like to point out that whatever the mechanism which brought about the increased secretion in experiment 1—rise of irritability of the nervous or glandular elements, or even a vascular mechanism—it had no relation to the pressing out of stagnant saliva from the ducts.)

Perfusion of the ducts. The first problem we tried to solve was to determine the rôle played by Wharton's duct and its chief divisions in the process of expressing saliva from the gland. To gather further data we perfused the dog's submaxillary ducts, in situ, with saline or Ringer solution at body temperature.

The data concerning the muscular elements of the salivary ducts and the contractile elements of the gland itself are rather confusing. (Vide literature by Mathews (5), Flint (9) and Metzner (10).) However, these data show that, probably, there is no lack of muscular and other contractile elements in the salivary glands.

The disposition of the ducts can be seen from figure 1, a reproduction of a skiagram of the dog's submaxillary gland injected with Beck's paste. (Bismuth subnitrate (B. W. & Co.)—50 parts in paraf. mol. flav (Cheseborough)—100 parts.) Our thanks are due to Dr. S. R. Johnston, director of the X-Ray Department of the Victoria General Hospital, Halifax, for a series of roentgenograms.

One cannula was inserted as usual in Wharton's duct near its buccal orifice. The second, smaller cannula was introduced into one of the chief divisions of the main duct (*A* on fig. 1). To keep the temperature of the

perfusing fluid constant at 37°C. the supply bottle of saline or Ringer solution was placed in a Dale's bath. From there the fluid passed through a tube connected with the smaller salivary cannula. This tube was wound, externally, with a wire, connected up to a transformer and a rheostat. The assembled apparatus was enclosed in a glass jacket filled with water.

Four experiments with perfusion of the salivary duct gave analogous results. Stimulation of the sympathetic nerve, sufficiently strong to produce an augmented secretion, had no effect on the rate of flow of the perfusing fluid. Stimulation of the chorda tympani increased the amount of fluid passing through the cannula by adding the secreted saliva to it. Subsequent stimulation of the sympathetic increased the number of drops falling from the cannula. There is no doubt that in all these cases saliva was admixed to the perfusing fluid, because the outflow became viscid. Therefore, this phenomenon is not due to the constriction of Wharton's

Fig. 1. X-ray photograph of the dog's submaxillary gland, injected through Wharton's duct with Beck's paste.

duct nor of its main branches. As an example we quote the following experiment.

Expt. 2. Dog. Chloralose. Perfusion of submaxillary ducts in situ, with 0.9 per cent saline at 37°C. Each figure represents the number of drops registered by the drop recorder in 30 seconds.

7, 7, 8 (Sy. C = 9½), 7, 7, 7, 7, 31 (Ch. C = 10), 10, 12, 10, 8, 8, 7, 7, 7, 10 (Sy. C = 9½), 8 (Sy. ditto), 7, 7.

Intravenous injection of atropin sulphate (5 mgm.) paralyzed the chorda tympani but did not change the effect of stimulation of the sympathetic nerve on the rate of the perfusing fluid. Neither did the addition to the perfusing fluid of adrenalin (Parke, Davis & Co., 1 to 2 cc. 1:10.000 and 1:1.000, injected into the rubber tube connecting the perfusing tube with the cannula inserted into the branch of Wharton's duct), nor pituitrin (Burroughs, Wellcome & Co., 1 to 2 cc. of 1:10.000, 1:1.000 and without dilution), change the rate of flow of the fluid through the ducts.

Blowing through and filling of Wharton's duct. Analogous results were obtained by blowing the saliva out of Wharton's duct between chorda and sympathetic stimulation, or by filling it with saline. The augmented effect of sympathetic stimulation did not disappear after the first procedure. The effect of stimulation of the nerve could not be replaced by filling the duct with saline. The following are suitable examples.

Expt. 3. Dog, as usual. Two cannulae, one inserted into Wharton's duct and one into one of its branches as described in text. Each nerve stimulated for 30 seconds. 4:00 p.m.—Ch., C = 10, 84 divisions. 4:04 p.m. Sy., C = 7, 20 div. 4:10 p.m.—Ch., C = 10, 57 div. Saliva then blown out from the duct. 4:14 p.m.—Sy., C = 7, 28 div. 4:24 p.m. Sy., C = 7, 5 div.

Expt. 4. Dog. Same procedure as in experiment 3.

3:22 p.m.—20 minims of saline injected into the duct. 3:23 p.m. Sy., C = 9, 4 div. 3:33 p.m. Ch., C = 10, 15 div. 3:35 p.m. 20 minims of saline injected into the duct. 3:36 p.m. Sy., C = 9, 10 div.

The last type of experiment, used in an effort to eliminate the influence of any contraction of Wharton's duct, consisted in the introduction of a long glass cannula, which reached almost to the hilus, into the duct of a cat's submaxillary gland. This experiment will be fully discussed below, in connection with the tracings which we obtained registering the movement of saliva under different conditions. At this juncture we would like to point out that in spite of this arrangement the augmented sympathetic secretion still persisted.

From these data we may conclude that the large salivary ducts of the submaxillary gland of the dog and cat do not play a part in the phenomenon of augmented sympathetic secretion. If during stimulation of the sympathetic nerve there is an expression of saliva from the gland we must infer that this is due to the contraction of smaller ducts or of the ampullae themselves.

Volumetric curves of chorda and sympathetic secretion. In order to obtain more precise data concerning the secretory and motor phenomena in the salivary glands we turned to the study of the volume changes of the saliva secreted under chorda tympani or sympathetic stimulation. Our earlier experiments were performed with a mercury manometer, through a system of tubes connected with the cannula inserted in Wharton's duct. Later on, in order to eliminate the inertia of the mercury and the possibility of damming back saliva, we used an instrument which may be called "Voluminometer." This Voluminometer, built on the principle of Hutchinson's spirometer, consisted of a small container filled with oil. Through the oil passed a tube, connected by a system of tubes with Wharton's duct. Over this tube, in the oil, was immersed a glass bell, the displacement of which changed the position of an equilibrated liver connected with it. In this apparatus the factor of back pressure was eliminated as far as possible for that type of instrument.

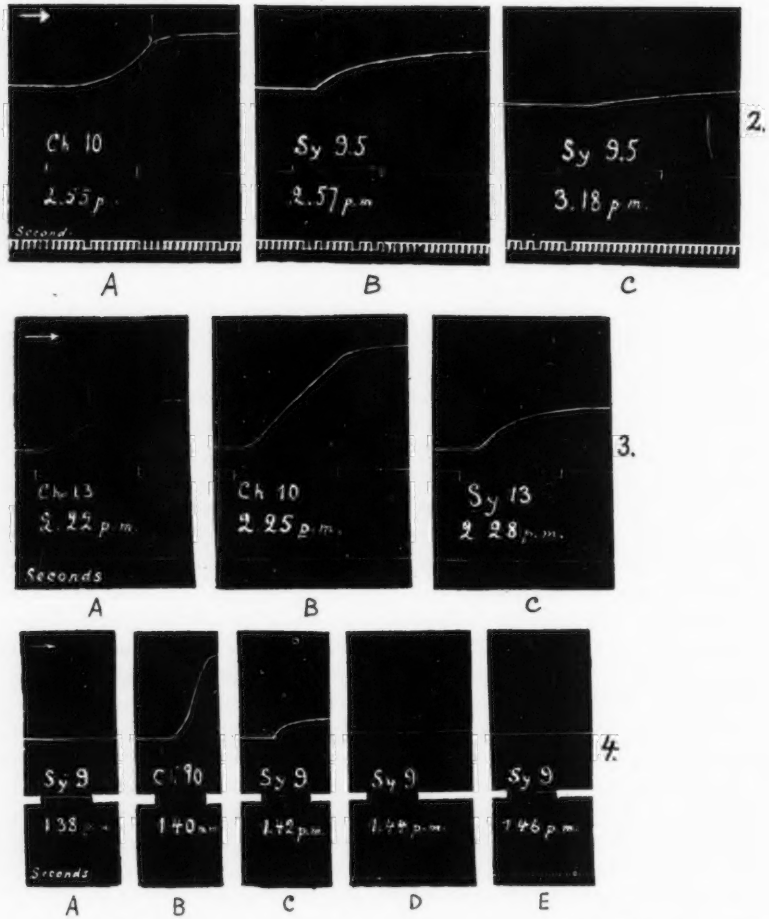

Fig. 2. Dog. Mercury manometer. A—Stimulation of chorda tympani. B—Stimulation of sympathetic nerve after chorda tympani. C—Stimulation of Sy. without previous stimulation of chorda tympani.

Fig. 3. Cat. Voluminometer. A—Stimulation of chorda tympani. Coil at 13 cm. B—Ditto. Coil at 10 cm. C—Stimulation of sympathetic nerve. Coil at 13 cm.

Fig. 4. Cat. A—Subliminal stimulation of sympathetic nerve. Coil at 9 cm. B—Stimulation of chorda tympani. Coil at 10 cm. C, D and E—Three successive stimulations of the sympathetic nerve. (Note that in D the secretion started after the end of the stimulation.)

The curves obtained by means of either apparatus were, however, very similar, probably due to the great pressure under which the saliva is secreted. The curves obtained after the stimulation of the chorda tympani, sympathetic and sympathetic after chorda, are not only different but also highly typical for each nerve and condition of the gland in the dog (fig. 2) as well as in the cat (fig. 3).

It is interesting to note that in the last experiment (fig. 3) the two chorda curves, in spite of the unequal strength of stimulus applied to the nerve, have the same character. The sympathetic curve has a quite different shape.

There is little doubt that we have here to deal with different processes occurring in the gland as a result of the stimulation of different nerves. In the case of chorda stimulation in the dog, as well as in the cat, the curve depicts a gradual, though more or less quick, filling of the ducts with secreted saliva. When the sympathetic nerve is stimulated the shape of the curve depends upon the amount of fluid contained in the ducts before stimulation and upon the rapidity with which the saliva is secreted. If there be none in the ducts, then, in the dog, whose sympathetic secretion is scanty and viscid, we see a slow, gradual rise of the lever of the recording apparatus. If the ducts be filled with saliva from previous stimulation of the chorda tympani we obtain a typical curve of augmented secretion. In the cat, very often, the primary stimulation of the sympathetic nerve gives a curve of the latter type. This is due to the fact that sympathetic stimulation in the cat provokes a rapid flow of thin saliva, i.e., the ducts are rapidly filled with fluid. (In some of the experiments on the cat, when sympathetic stimulation was not fully effective, we could see, as in the dog, a gradual slow rise of the curve.)

The repeated stimulation of the sympathetic after previous stimulation of the chorda tympani, in a dog, gives, first, a typical augmented sympathetic curve, then, a curve with a slow rise. In the cat an analogous phenomenon may be obtained if subliminal stimulation be applied to the sympathetic nerve, as is seen in figure 4.

The mathematical expression of these curves, i.e., the derivation of rate curves from volume-time curves, even more strongly emphasizes their different character. (Our thanks are due to Prof. G. H. Henderson and Mr. W. G. Moran, of the Dept. of Physics of Dalhousie University, who helped us with derivation of these curves.) As an example we give here the curves on figure 5, which represent the volume curves of chorda tympani and sympathetic nerves, from cat's submaxillary gland, and derived from them the rate curves.

Mechanical schemas. We have made an attempt to analyze the data obtained from this study by using simple mechanical schemas. One of

such schemas was arranged in the following way. A rubber balloon, filled with water, and connected by means of tubes with the voluminometer, was enclosed in a bottle containing water. This latter bottle was connected by tubing with a pressure bottle, also containing water. By raising the pressure bottle, the pressure on the balloon enclosed in the first bottle could be increased. This schema showed us that the shape of the voluminometer curve depends, not on the amount of fluid (unless it be very small) entering the tube connected with the registering apparatus, but on the time in which a certain amount of fluid enters the tube, i.e., it depends on the rate of inflow of the fluid. The curves obtained by means

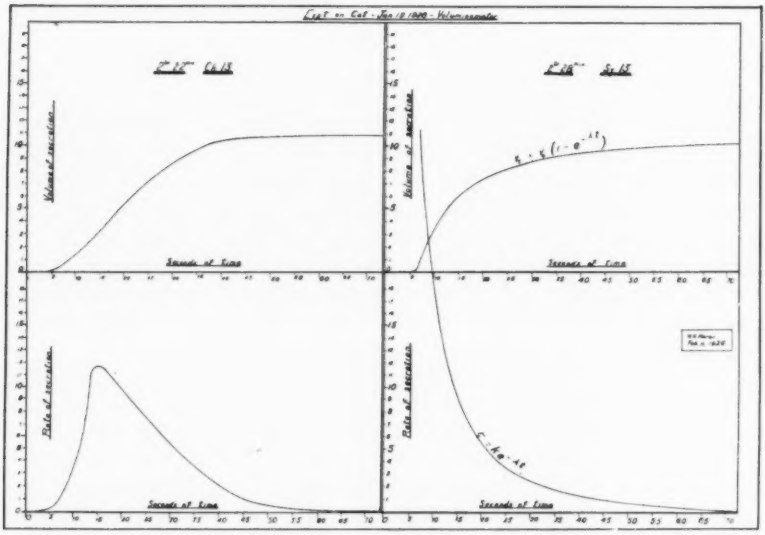

Fig. 5. Submaxillary gland of a cat. Voluminometer

of this schema under different conditions were analogous to the corresponding volumetric curves of the salivary secretion.

The sympathetic nerve alone, possesses the property of expressing the fluid from the gland. Mathews (5) and Anrep (6) have demonstrated that atropin does not paralyze the motor fibres of the sympathetic nerve in the dog. A fluid injected back into the ducts of an atropinized dog is pressed out from the gland by stimulation of the sympathetic nerve. We could confirm this statement and show that, whereas under these conditions stimulation of the chorda tympani was ineffective, the stimulation of the sympathetic gave a positive mechanical effect. Besides this we could demonstrate that the voluminometer curves of sympathetic

stimulation in the cat, before and after atropinisation of the animal, presented practically the same shape (see fig. 6).

The slight deformation in the middle of the second curve is due to accidental movement of the recording lever of the voluminometer.

Experiment of insertion of a deep cannula into Wharton's duct. Evidence has been adduced above to the effect that the main duct plays no part in expressing the saliva. We now present a further and different type of experiment confirming this statement.

Expt. 5. A long, thin, glass cannula was inserted into the duct of Wharton in a cat for a distance of 38 mm. The total length of the duct from the site of insertion of this cannula to the hilus was 42 mm. The open end of the cannula was connected with the voluminometer. The shape of the chorda tympani curve and augmented sympathetic curve remained practically unchanged under this arrangement.

Discussion. The following conclusions may be drawn from the data presented above.

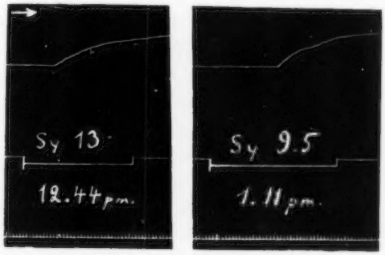

Fig. 6. Cat. 12:44 p.m., volume curve of the sympathetic secretion. 12:50 p.m., 0.001 gram atropine sulphate injected intravenously. Ch. tympani became paralysed. Stimulation of Sy. (coil—9.5 cm.) gave a scarcely perceptible secretion. 1:11 p.m., 0.25 cc. of saline blown back into the gland and Sy. stimulated (coil—9.5 cm.).

The volume-time curves and, derived from them, the rate curves of salivary secretion show that different processes may occur in the submaxillary gland under the influence of different nerves. In one case we have a gradual rise of the curve, which corresponds to the more or less rapid filling of the ducts with the secreted saliva (chorda tympani and the secretory fibres of the sympathetic nerve). In the other case the saliva already accumulated in the ducts (dog), or rapidly secreted (cat) is pressed out from the gland (motor fibres of the sympathetic nerve). This last phenomenon plays an important part in the process of the augmented sympathetic secretion.

Our data, however, show that it would not be quite accurate to explain the whole phenomenon of augmented sympathetic secretion as due to mechanical process only. Against this supposition are presented the experiments with repeated stimulation of the sympathetic nerve in a dog (expt. 1), when, in spite of massage of the gland, the secretion was increased by subsequent stimulations of the nerve. The same interpretation may be drawn from the experiments in which subliminal stimulation was applied to the sympathetic nerve in a cat (see fig. 4). This stimulation was ineffective before stimulation of the chorda tympani. It gave a

typical "augmented sympathetic" curve immediately after the action of the parasympathetic nerve. The second stimulation of the sympathetic nerve gave a "secretory" sympathetic curve. The third stimulation was again ineffective. Therefore, we have reason to refer to a "true" augmented secretion, due to increased secretory response of the gland after its previous stimulation.

There are other phenomena we may regard from this point of view. Thus the augmented effect of chorda tympani after chorda tympani stimulation must be included in this category, because there is no ground admitting the existence in the chorda tympani nerve of motor fibres. The same must be said regarding the inconstant duration of the after-effect of chorda or sympathetic stimulation. In some experiments, for unknown reasons, the after-effect of a moderate stimulation of the nerve may last, instead of the usual 1 to 2 minutes, as long as 20 to 30 minutes. It would appear that the same nervous impulse may, under certain circumstances, meet in the gland special conditions which greatly facilitate its influence upon the secretory elements of the gland.

Therefore we think it would be more in keeping with the real state of affairs to admit that there are two phases in the augmented sympathetic secretion,—a mechanical and a secretory.

SUMMARY

1. An augmented secretory effect from stimulation of the sympathetic nerve after previous stimulation of the same nerve and massage of the submaxillary gland in a dog, was demonstrated.

2. Contraction of the ductus submaxillaris, and its chief divisions, in the dog as well as in the cat, is excluded as causative of the phenomenon of the augmented sympathetic secretion.

3. The volume-time curves and, derived from them, the rate curves of the salivary secretion show that different processes,—secretory and motor—may occur in the submaxillary gland under stimulation of the chorda tympani and sympathetic nerve.

4. The chorda tympani nerve, of a dog and a cat, supplies the secretory elements of the submaxillary gland with secretory fibres. The sympathetic nerve in both animals contains secretory and also motor fibres for the contractile elements of the gland.

5. A view is advanced that there are two phases in the augmented sympathetic (after chorda) secretion,—a mechanical phase, due to the action of motor fibres in the sympathetic nerve and a secretory phase which appears as a result of previous stimulation of the parasympathetic nerve.

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THE SPLANCHNIC NERVE AS A SECRETORY NERVE OF THE GASTRIC GLANDS

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The secretory influence of the vagus nerve on the gastric glands is a well-established fact. But there is no direct evidence that the second pair of nerves which supplies the stomach—the splanchnics—have a direct relation to the function of these glands. The only indication that the sympathetic nervous system may influence the gastric secretion is the experiments with positive secretory effect of adrenalin (1), (2), (3).

The following investigations are to demonstrate that the electrical stimulation of the peripheral end of the splanchnic nerve provokes gastric secretion. Owing to the presence of vaso-constrictor fibres in the splanchnics and possibly of special "secretory-inhibitory" fibres to the gastric glands, the following series of experiments were devised.

1. Stimulation of the splanchnic nerve in a spinal dog in an "acute" experiment. (Ushakow (4), in Pavlov's laboratory, used this method for the demonstration of vagus action on the gastric glands.)

2. Stimulation of the splanchnic nerves sectioned five days before the experiment. A spinal preparation was also used in this case. We expected that during this period the vaso-constrictor fibres, the hypothetical "secretory-inhibitory" fibres and the secretory fibres to the adrenal glands would have degenerated. (This method was applied to the vagus nerve by Pavlov and Schumova Simanovskaja (5) to detect the secretory action of this nerve on the gastric glands, and by Kudrevetzky (6) to the splanchnic nerve to show the secretory influence of the sympathetic nerve on the pancreas.)

3. Stimulation of the splanchnic nerve sectioned five days before the experiment, in a dog with Pavlov's or Heidenhain's pouch, without the use of anesthetics.

First group of experiments. Under ether-chloroform anesthesia, we started by cutting the spinal cord just below the medulla oblongata. A tracheotomy was performed for artificial respiration. To stop the inhibitory impulses which could be transmitted to the gastric glands through the pneumogastric nerves, both were cut Loss of water, due to flow of saliva, after cutting the spinal cord, occasioned unfavourable conditions for the

work of the gastric glands (4). We arrested this secretion by sectioning the two chorda tympani. The esophagus was tied in the neck, to prevent the mixing of swallowed masses with the contents of the stomach. The cardiac part of the stomach on the boundary with pylorus was sewed subserously and tied, so that the passage to the pylorus was tightly closed. A gastric fistula tube was fixed in the fundus. Thereafter, the abdominal wound was carefully closed, with only the fistula protruding. After this operation the animal was placed back upwards and, entering from the back, both splanchnics were reached by the retro-peritoneal route and enclosed in shielded electrodes. Following these preliminary operations the dog was suspended by broad straps in a frame, in a position corresponding to a standing dog and surrounded with warming flasks. The stomach was washed with tepid water.

The splanchnic nerves, thus prepared, were stimulated by means of a rhythmic tetanisation. Half hours of stimulation and rest were alternated. During the half-hour period of stimulation the right and left nerves were alternately excited fifteen minutes each. This was repeated for three or more hours. The liquid flowing from the gastric fistula, if in sufficient quantity, was measured and tested for HCl and for its digestive power.

Experiments 1 and 2 present examples of such experiments.

Experiment 1. Dog, prepared as described above. The left splanchnic nerve in shielded electrodes, the right stimulated by ordinary electrodes.

TIME	GASTRIC JUICE	SPLANCHNIC NERVES
minutes	*cc.*	
30	1.0	Stimulation
6	0	Rest
30	1.5	Stimulation
45	0.7	Rest
30	1.2	Stimulation
30	3.6	Stimulation

Experiment 2. Dog. Both splanchnics are in shield-electrodes.

TIME	GASTRIC JUICE	SPLANCHNIC NERVES
minutes	*cc.*	
30	0.4	Stimulation
30	0.1	Rest
30	0.6	Stimulation
15	0.4	Rest
30	2.5	Stimulation
30	0.4	Rest

As may be seen from these experiments the gastric glands, after a few periods of excitation, respond distinctly to the stimulation of the splanchnics.

Second group of experiments. Aiming to obtain a stronger action from the stimulation of the splanchnics, we tried in another set of experiments to excite nerves which had partly degenerated after previous section. In our experiments we followed closely the method indicated by Kudrevetzky (6). In an aseptic operation we sought, by the retroperitoneal route, the left splanchnic nerve, which we cut as closely as possible to the diaphragm and, securing it by a ligature, closed the wound. Five days later the "acute" experiment was performed. The left splanchnic nerve was found in the wound and put in the electrodes. The right splanchnic nerve was then also cut and also placed in the electrodes. The results are shown in experiments 3 and 4.

Experiment 3. Dog. March 20, 11 a.m., left splanchnic cut and ligatured. On March 25 from 11:30 a.m. to 12 m. preparation of the animal as in experiment 1 and 2. Blood pressure recorded by cannula in the femoral artery. Both splanchnics in shield-electrodes. During the stimulation the left and the right splanchnic nerves were excited alternately as in experiment 1.

TIME	GASTRIC JUICE	SPLANCHNIC NERVES
minutes	*cc.*	
20	0	Rest
30	0.6	Stimulation
30	0	Rest
30	0.9	Stimulation
30	2.3	Stimulation
30	0.2	Rest
30	3.2	Stimulation
40	0.3	Rest
40	1.6	Stimulation
15	0	Rest
40	1.0	Stimulation

Experiment 4. Dog. Five days before the experiment section of the left splanchnic nerve. Only this nerve stimulated.

TIME	GASTRIC JUICE	LEFT SPLANCHNIC NERVE
minutes	*cc.*	
30	0	Stimulation
35	1 drop	Rest
20	0.1	Stimulation
50	1 drop	Rest
40	2.2	Stimulation
40	0.5	Rest
45	1.4	Stimulation
35	0.2	Rest

The secretory influence of the splanchnics in this cases was quite marked. In experiment 3 we recorded the blood pressure in the femoral artery. It is interesting to note that the excitation of the partly degenerated nerve did not induce any change in the blood pressure. This shows that on the day of the experiment (the fifth after cutting the nerve) the nerve not only had lost its direct vaso-constrictor influence, but also failed to provoke any increase in the output of adrenalin. The secretory sympathetic fibres to the gastric glands were still active. The freshly cut right splanchnic nerve gave on stimulation the usual rise of blood pressure.

Third group of experiments. The preliminary operations described above occupied so much time and debilitated the animal to such an extent that we could not expect a normal response from the gastric glands on stimulation of the splanchnic nerves, though we observed in all the experiments a distinct connection between excitation and secretion.

Prof. I. P. Pavlov suggested that we reproduce with the splanchnic nerve, in a "semi-acute" experiment, those conditions which had first enabled him to overcome the inhibitory influence of the vagus nerve in relation to the gastric glands.

To make sure that one is working with the fundus glands, we used dogs with Pavlov's or Heidenhain's pouch. On such an animal, five days before the experiment, the left splanchnic was cut as described above. On the day of the experiment the dog was put in the frame, making certain the gastric glands were at rest. Thereupon under primary chloroform anesthesia the wound on the back was opened, the left splanchnic secured and placed in shield-electrodes. The wound was then sewed up with one end of the electrodes protruding. This operation was performed under strict asepsis. Further precautions were taken to prevent the escape of current. The anesthethic was then arrested and the dog replaced in the frame. At this stage care must be taken to prevent any movements which might displace the electrodes. Such movements, however, soon cease. All preparations for the experiment took approximately an hour.

Up to the present time we have carried on three experiments in this manner. The first experiment on a dog with a Pavlov's pouch proved a failure. We have reason to suppose that the stimulation of the splanchnics could not overcome the inhibitory influence of the vagus nerve. We decided, therefore, to use for these experiments only dogs with Heidenhain's pouch. Two experiments of this kind were performed; experiment 5 shows the results of one of them.

Experiment 5. Dog with Heidenhain's stomach pouch. April 5, in the evening, left splanchnic nerve sectioned by the retroperitoneal route. On the day of the experiment, April 11, in the morning the dog was placed in the frame. In the course of an hour a few drops of mucus of acid reaction flowed out of the pouch. At 12:05 p.m. the operation for placing the splanchnic nerve in the electrodes began. At

1 p.m. the animal was replaced in the frame. Copious salivation which soon stopped. In the first minutes after awakening the dog made an attempt to lick the wound on the back, then stood still.

Secretion from the pouch.

From 1:00 p.m. till 1:30 p.m.—0.4 cc. Acid mucus, no free HCl
From 1:30 p.m. till 1:45 p.m.—0.1 cc.
From 1:45 p.m. till 2:00 p.m.—0.2 cc.
From 2:00 p.m. till 2:25 p.m.— 0 Neutral reaction in the pouch

The excitation of the left splanchnic nerve by rhythmic tetanisation began at 2:25 p.m.

TIME	SECRETION FROM		LEFT SPLANCHIC NERVE	COIL	JUICE FROM POUCH	
	Pouch	Main stomach			Reaction	Free HCl
	cc.	*cc.*		*cm.*		
2:55 p.m.	3.5		Stimulation	24–21	Acid	Present
3:10 p.m.	0.2		Rest			None
3:30 p.m.	0.4		Stimulation	21	Acid	
3:45 p.m.	1 drop		Rest			
4:15 p.m.	0.4	10.0	Stimulation	15		
4:30 p.m.	0.2	2 drops	Rest			
5:15 p.m.	0.6	3.0	Stimulation	14*		
5:30 p.m.	0.2		Rest		Acid	None
5:45 p.m.	0	1.0	Rest			
6:00 p.m.	0.8		Stimulation	11	Acid	Present
6:15 p.m.	0.4	6.0	Stimulation	11	Acid	Present
6:30 p.m.	0.3	5.0	Stimulation	10	Acid	Present

* Latent period 5 minutes.

As one can see, the experiment in this form gave results fully confirming our former conclusion.

The second experiment performed on a dog with Heidenhain's pouch did not yield such striking results, nevertheless it gave clear-cut confirmation of the secretory significance of the splanchnic nerve.

On the basis of the above experiments we believe we have a right to state that besides the pneumogastric nerves, the sympathetic nervous system through the splanchnic nerves also conveys excitatory secretory fibres to the fundus glands of the stomach.

SUMMARY

Several forms of experiments were devised by means of which it was demonstrated that the splanchnic nerves contain secretory fibres for the gastric glands.

Our thanks are due to Prof. I. P. Pavlov for his advice and criticism during this work.

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SUDDEN, TRANSITORY REDUCTION IN THE VISCOSITY OF THE BLOOD AS A CAUSE OF THE FALL IN BLOOD PRESSURE IN "SHOCK"

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Accompanying the fall in blood pressure in peptone, histamine and anaphylactic "shock," there has been found a transitory but marked reduction in the viscosity of the blood (Waud, 1926). As the mechanisms of the fall in blood pressure in anaphylactic, peptone and histamine "shock" seem very similar and as no great differences between them have been demonstrated, no attempt is made in this paper to consider these conditions separately.

Previously advanced theories. Various theories have been advanced as an explanation of the fall in blood pressure accompanying the above conditions. Biedl and Kraus (1909) in their early work on anaphylactic shock stated that the fall in blood pressure was due to a reduction in the peripheral resistance brought about by a paralysis of the vasomotor mechanism. They based their conclusions on the fact that the fall in blood pressure following the injection of horse serum or other foreign protein may be prevented by the injection of barium chloride, which, by stimulating the smooth muscle of the arterioles, counteracts the depressor action of the shock-producing substances. Simons (1919) attributes the congestion in anaphylactic shock to spasm of the hepatic veins and bases his theory on the finding of unusually well-developed muscle in these veins. Airila (1914) is of the opinion that the fall in blood pressure in rabbits is due to an increased resistance in the pulmonary vessels. Dale (1918) has produced indirect evidence that the fall in blood pressure in histamine shock is brought about by an active dilatation of the capillaries. Hooker (1920), by observing the rabbit's ear under the low power microscope, found that in histamine shock not only did the capillaries dilate but, also, the veins leading from them became filled with blood and definitely dilated. Manwaring (1924) has shown that if the liver is excluded by means of an Eck fistula anaphylaxis does not occur in dogs. The present writer (1926) conceived the idea that the fall in blood pressure in peptone, histamine and anaphylactic "shock" might be due to a diminished viscosity of the blood

with consequent lowering of the peripheral resistance. This hypothesis is supported by the observations to be reported in this article, but before describing these a brief résumé will be given of the phenomena of these shock conditions.

Characteristic features of shock. The most characteristic feature of anaphylactic and peptone "shock" is a marked fall in arterial blood pressure. This decreased arterial pressure is accompanied by an accumulation of blood in the liver and the large veins of the abdomen with a consequent rise of venous pressure. This condition is described by Pearce and Eisenbrey (1910) as a "bleeding into the veins of the abdomen." Manwaring (1922) found the portal pressure increased from a normal of 9 mm. Hg to about 19 mm. Hg in anaphylactic shock, the maximum being reached at the end of one minute; the portal pressure then gradually falls and is restored to normal in 8 to 15 minutes. In peptone and anaphylactic shock the flow of lymph is definitely increased, and its coagulability is first increased and then decreased (Kmietowixz, 1924). Symptoms of contraction of the smooth muscle of the bronchioles are very marked especially in the guinea pig. Evacuation of the bowels followed by bloody diarrhea is always present in severe anaphylactic shock in dogs; salivation and vomiting are also frequent symptoms in that animal.

Post-mortem findings in shock. The post-mortem findings in anaphylaxis in dogs as described by Dean and Webb (1924) are as follows: the sinusoids in the liver show marked dilatation and hemorrhages are evident beneath the capsule and about the central veins. Hemorrhages into the gall bladder, intestine and stomach are evident. The lung, particularly in the guinea pig, is markedly distended, a condition attributed to a constriction of the smooth muscle of the bronchi, which constriction prevents escape of air from the alveoli. In view of the fact that this distention is maintained after death, Schmidt and Barth (1923) attribute it to an edema of the lungs rather than an impounding of air in the alveoli.

EXPERIMENTAL METHODS. In the work to be described in this paper three groups of experiments were performed. First the viscosity of the blood of rabbits was determined before and after the injection of peptones. Second, the same observations on rabbits were made before and after the injection of histamine. Third, viscosity of the blood of dogs was determined before the injection of a sensitizing dose of antigen, during the process of sensitization, just before the injection of the provocative dose of antigen, following the injection of antigen and for several days thereafter.

The precise technic in rabbits was as follows: Rabbits were anesthetised by an intraperitoneal injection of 20 cc. of a 10 per cent solution of urethane per kilo of body weight. Blood pressure was recorded in the carotid artery; blood for the determinations was withdrawn by means of a cannula in the femoral artery; peptone or histamine solution was injected into the ex-

ternal jugular vein. Viscosity determinations were made by means of a viscosimeter of the Oswald type. This consists of a glass U-tube one arm of which is of capillary bore, the upper end of the capillary expanding into a bulb with a capacity of approximately 5 cc. The viscosity was determined by the time taken for the 5 cc. of blood to flow downward from the bulb through the capillary tube under the influence of gravity. When

TABLE 1

Summarizing the changes in the viscosity of the blood of rabbits, following the injection of 0.5 gram of peptone per kilo of body weight

RABBIT NUMBER	VISCOSITY BEFORE INJECTION (AQUA-1)	VISCOSITY AFTER INJECTION (AQUA-1)	PER CENT REDUCTION IN VISCOSITY
	Peptone in rabbits		
1	5.3	4.0	25
2	4.5	3.8	14
3	5.0	4.0	20
4	3.5	2.5	28
5	5.3	4.5	25
6	4.1	3.2	16
7	4.7	3.2	28
8	3.6	2.8	21
9	3.6	3.1	14
10	3.5	2.6	14
11	2.0	1.4	16
12	2.3	1.6	22
13	4.3	2.6	38
14	2.5	2.1	13
15	2.6	2.1	24
16	4.5	2.5	44
17	3.4	2.3	31
18	3.5	2.7	22
19	3.5	2.5	28
	Saline only in rabbits		
20	3.2	3.1	3
21	3.5	3.4	2

thoroughly dried the viscosimeter was placed in a water bath, the temperature of which was kept constantly at 37°C. by means of a toluene electric thermostat.

Two different anticoagulants were used, sodium oxalate and "heparin" of Howell (1918). Although in about 15 minutes after the injection of peptone the blood usually becomes incoagulable, it was found in a great number of cases that immediately following the injection of this substance the coagulability is greatly increased and unless large amounts of oxalate

(0.2 gram in 6 cc. of blood) were used and the blood on withdrawal well mixed but not shaken with it, there would be formed small threads of fibrin almost microscopic in size which interfere with the passage of blood through the capillary tube, thus obscuring the true result of the experiment. Heparin, although required in larger quantities than usually described, was found to be a very efficient anticoagulant. It was found that this initial tendency to increased coagulability of the blood could be considerably reduced by filtration of the peptone solution before injection. It was also found necessary to make sure that the cannula, by means of which the blood is withdrawn, was well washed with an anticoagulant ($\frac{1}{2}$ saturated solution of sodium sulphate) before and following the withdrawal of the blood, the sulphate being removed by letting a little blood escape at the beginning of the withdrawal. Blood was withdrawn and its viscosity determined as a "normal" before injection of the peptone. The quantity of peptone injected was that ordinarily used to produce incoagulability of the blood, which according to Stewart (1918) is 0.5 gram per kilo of body weight; this was dissolved in 10 cc. of 0.9 per cent NaCl solution, the viscosity of which in control experiments had been brought up to that of blood by addition of gum acacia. An important point in the technique is the time at which the blood is withdrawn. It was found that the maximum fall in viscosity was obtained when the withdrawal of the blood was commenced at the time when the peptone solution was about half injected and continued until a few seconds after the completion of the injection. It appears that this change in fluidity of the blood takes place in about one complete circulation time which, according to Stewart (1894), in a 2 kilo rabbit is about 10.5 seconds. The viscosity determinations were made immediately upon withdrawal of the blood, before sedimentation of the corpuscles had taken place.

In order to exclude the possibility of the reduction in viscosity being due to the withdrawal of the amount of blood required for the determination of the "normal" or to the saline in which the peptone was dissolved, experiments were performed in which 10 cc. of 0.9 per cent NaCl solution were injected without the peptone.

Results. The results of the above experiments are shown in the accompanying table. It will be noted that during and immediately following the injection of peptone there is a reduction of from 14 to 44 per cent in the viscosity of the blood. That the fall in viscosity of the blood is really due to the peptone is proved by the observation that injection of saline, without peptone, causes a reduction of only 3 per cent in viscosity.

A few experiments were performed in which histamine was injected into a rabbit, the viscosity being determined before and after the injection; it was found that although the reduction in viscosity was not so marked as in the peptone experiments, nevertheless the results are sufficient to justify further investigation along this line.

Methods employed in dogs. In the experiments in which 15 dogs were sensitized with antigen, the following procedure was followed. After determining the viscosity of the dog's blood once or twice a day for three days, 5 cc. of horse serum were injected into the external saphenous vein.

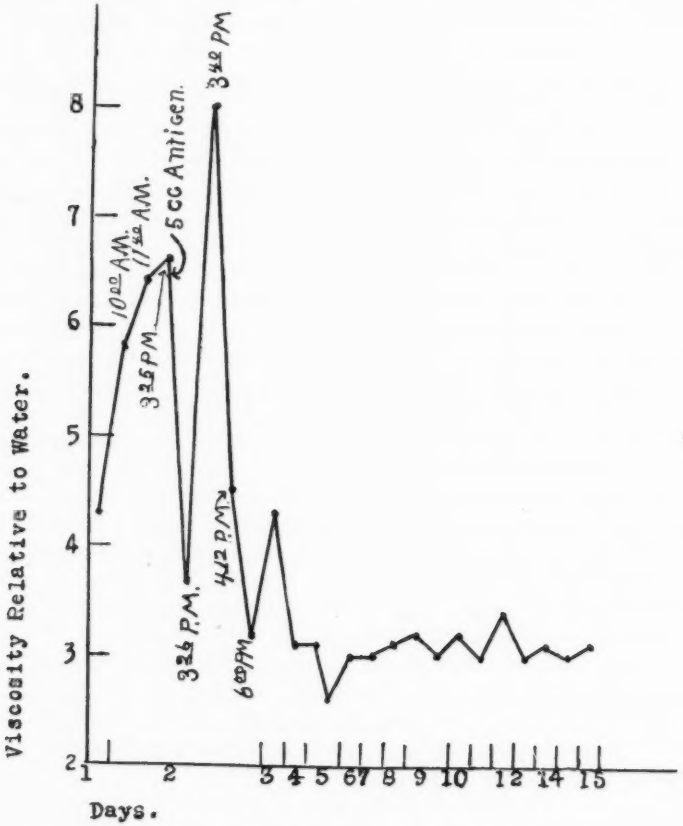

Fig. 1. Changes in the viscosity of the blood of a dog on injection of the provocative dose of antigen. The animal was given the sensitizing dose about four weeks previously.

Viscosity determinations were then made daily. On the twenty-second day the animal was anesthetised with ether and arranged for recording blood pressure, the withdrawal of blood and injection of antigen. In some experiments the blood pressure was not recorded, the animal being placed

on the table, and the provocative dose of antigen injected into the right saphenous vein, the blood for viscosity determinations being withdrawn from the left leg. It was found necessary to observe even more strict precautions as to the amount of anticoagulant, the time of withdrawal of blood, etc., than were found necessary in the peptone experiments. Viscosity determinations were made for several days following the recovery from shock.

Results of the above experiments on dogs. A typical result of the above experiments is shown in the chart. During the stage of sensitisation there was no apparent deviation from the normal viscosity until the time was reached at which a second dose was likely to give definite symptoms of shock. At this time the variations in viscosity became somewhat greater, and occurred during much shorter intervals of time. Variations which normally involved several hours took place in less than half an hour; in three dogs of the series this was very noticeable. Blood drawn at the completion of one circulation time following the injection of the provocative dose of antigen showed a marked fall in viscosity. This condition of low viscosity is very fleeting and if the blood is not obtained at the proper time will pass unnoticed, for immediately following, as is seen in the chart, the blood not only returns to the viscosity prevailing before the injection of serum, but reaches a much higher level. In one animal the blood was so viscous that it would barely pass through the capillary tube of the viscosimeter, yet on examination there were no signs of fibrin formation. As the animal recovers, this increase in viscosity passes off. Although the fluidity of the blood is somewhat unstable for the following 24 hours, after this time there is a definite stability of the viscosity, there being practically no variations during the following six days at which time determinations were discontinued.

Discussion. That a reduction in the viscosity of the blood causes a fall in blood pressure was shown by Bayliss (1917). This author showed that a 30 per cent reduction in the viscosity of the blood, produced by a withdrawal of blood and its replacement by an equal amount of saline, is accompanied by the same percentage reduction in blood pressure. He also showed that in order to produce a 30 per cent reduction in the viscosity of the blood of a rabbit it is necessary to bleed the animal to the extent of 110 cc. and replace the blood by an equal amount of saline. It is therefore plausible to conclude that the reduction in the viscosity of the blood is sufficient to account for the greater part, if not all, of the fall in blood pressure in these conditions of shock.

It is evident that a decrease in viscosity of the blood will have an effect similar to that produced by dilatation of the arterioles, namely, an increased flow of blood through the arterioles into the capillaries and an increased pressure in the latter; and the fall in pressure from the arterioles to the

capillaries will be less than when the arterioles are constricted or the viscosity of the blood is high. If the tissue is distensible and elastic, the increased pressure in the capillaries will cause dilatation of these vessels. This dilatation would be especially evident when the increased blood flow had filled the available potential space in the veins, so that free escape of blood from the venous side of the capillary bed is prevented. It is evident that the rate at which any changes in the peripheral resistance take place will be an important factor. When the rate of change is a gradual process the other factors which are responsible for the maintenance of blood pressure come into play so as to compensate for changes in the peripheral resistance. Thus by such adjustments the blood pressure is kept at a constant level and the escape of too much blood from the arteries into the capillaries is prevented. If on the other hand a considerable decrease in viscosity were to take place suddenly so that the compensating factors would not have time to act, there would be a few beats of the heart whose force would be too great for the blood of low viscosity. For a few seconds the pressure in the capillaries and also the volume of blood flowing into them would be greatly increased. The capillaries are "blown up" with blood in a way which may be understood by a consideration of the following mechanical analogy. Consider a large syringe filled with some viscous substance such as molasses. In order to force any quantity of this substance through a needle of medium bore one would be required to exert considerable pressure on the piston of the syringe. If now changes were to take place in the colloidal state or the molecular arrangement of the substance in the syringe, such that its viscosity and thus the resistance in the needle of the syringe were suddenly reduced to nearly that of water, a large volume of the fluid would be suddenly forced out of the syringe. This sudden outflow would be due to the fact that the pressure exerted by the hand on the piston of the syringe could not be reduced with sufficient speed to compensate for the sudden reduction of the resistance in the needle. The force pressing on the piston of the syringe represents the force of the heart beat, the chamber of the syringe the arteries, the needle the arterioles and the space beyond the needle the capillary bed. In the same way consider the heart beating against a high peripheral resistance; if this peripheral resistance be suddenly decreased by a lowering in viscosity of the blood, the blood under high pressure in the arteries and subjected to the force of the strong beating heart would be allowed to enter the capillary area suddenly and in large volume so that instead of the pressure in the capillaries being maintained at a proper level it would be allowed to approach that of the large arteries. The capillaries for a short period are thus subjected to a pressure far higher than that which they are able to withstand and as a result they are injured beyond immediate repair and a large part of the blood required to maintain an effective circulation is retained in them.

In the chart it will be seen that following the fall in blood viscosity there is an immediate and marked rise in viscosity. During this period of high viscosity or internal friction it was noted that the symptoms of bronchial constriction were very marked. Gasser and Hill (1924) on comparing the resting with the excited muscle found that the contracted muscle is more viscous than the resting muscle. Changes in the viscosity of the cytoplasm of other cells as a result of stimulation have been demonstrated by Bayliss (1920). The cytoplasm of the amoeba contains many granules in active Brownian movement, and hence must be liquid: on stimulation the movement instantly ceases, suggesting that the cytoplasm has temporarily set to a state of gel. In view of the work of these authors it would be plausible to suggest that the increased viscosity following the initial decrease was not confined to the blood but extended to the smooth muscle substance and may be associated with the bronchial spasm.

As stated above, in many cases, at least, diminution in viscosity is transitory and there is a rebound of viscosity above the normal in spite of the fact that a fall in blood pressure is observed as a constant condition in "shock." This may offer an apparent difficulty in explaining the whole phase of the lowered blood pressure on the basis of viscosity changes. Although this diminution is transitory, it is of sufficient duration to allow a considerable part of the blood contained in the arteries to be forced into the capillaries. The capillary walls which are normally subjected to a pressure of 30 to 50 mm. Hg are, according to the theory advanced in this work, suddenly subjected to a pressure much above that; as a result they are markedly distended even to the point of rupture as was found by Dean (1924) on post-mortem examination. In all probability the capillary endothelium is injured beyond immediate repair. By the time the viscosity of the blood has returned to normal, as mentioned above, the condition in the capillaries is such that they are unable to contract normally and thus pass the large volume of blood which they contain on into the veins. The arterial blood pressure at this time is low and so gives little assistance to the flow of blood in the capillaries. Hence the blood which is required to make up sufficient blood volume in the large vessels is pooled in the capillaries. With this blood out of the current circulation any increase in viscosity will be of no avail in restoring the blood pressure, and only as this blood is transferred from the capillary area to the large vessels is the blood pressure restored.

In conditions of shock there is an increased flow of lymph, a fact already mentioned. According to the theory advanced in this work an increased pressure in the capillaries would no doubt be a factor in the passage of fluids out of the circulation and the resulting increased flow of lymph. However, since considerable evidence has been brought forward by Spaeth (1916) to show that a lowering of the viscosity of the cell surface results in

more rapid diffusion of substances through the cell, it would be plausible to conclude that the initial lowering of the viscosity of blood in conditions of shock may be associated with a lowering of the viscosity of the surface layer of the endothelial cells of the capillaries; thus allowing a more rapid passage of the fluid contents of the blood into the tissues, and thereby increasing the amount of fluid in them. These observations are strengthened by the observations of Petersen (1923). Petersen found an increase in proteins and globulins in the lymph following the injection of peptone and interpreted it as an indication of increased endothelial permeability. This increase in permeability, like the decrease in viscosity found in the present work, was reversible, that is, at the beginning of shock the viscosity of the blood was low and the permeability of the endothelial cells was increased. Later the viscosity of the blood was high and the permeability of the cells decreased. Both the initial factors would favor a passage of fluid out of the blood into the tissues and thus increase the edema.

Kyes and Strauser (1926) were able to prevent anaphylactic shock in pigeons by an intravenous injection of heparin twenty-five minutes previous to the giving of the provocative dose of antigen. In the summer of 1925 the writer, in collaboration with Dr. A. B. Luckhardt, performed similar experiments on guinea pigs. In our experiments all the heparinized animals showed symptoms of shock, the severity of which, in a great number of cases, was greater than that observed in the control animals. This discrepancy may possibly be explained by differences in the reactions of the blood of mammals and birds. Zuna (1925) observed that the ability of hirudin to prevent anaphylactic or peptone shock, was directly proportional to its ability to prevent the lowering of surface tension of the blood plasma. It is suggested that heparin may have a similar effect, either directly or indirectly, upon the viscosity of birds' blood.

The nature of the substance or mechanism whereby this decrease in viscosity is brought about, or the place of formation of such substance has not been considered; but in view of the work of Manwaring (1924), evidence points toward its formation in the liver.

SUMMARY

The theory is advanced that the fall in blood pressure in anaphylactic shock and allied conditions is brought about by a sudden transitory reduction in the viscosity of the blood. The reduction in viscosity causes the fall in blood pressure because of the fact that the less viscid blood passes through the small arterioles more readily, and thus the pressure and blood volume in the capillaries is suddenly increased.

The results of a series of experiments are reported in which viscosity determinations were made before and following the injection of peptone, histamine or antigen. These experiments support the above theory.

It was also noted that following the decrease in viscosity and accompanying the symptoms of bronchial constriction in the anaphylactic dog, there was a marked increase in the viscosity of the blood. It is suggested that this increase in viscosity extends to the muscle plasma and may be associated with the muscular contraction in the bronchi.

A reduction in the viscosity of the limiting membrane of the endothelial cells of the capillaries is mentioned as a probable contributing factor in the production of the edema in "shock."

In conclusion I wish to express my sincere appreciation to Drs. A. J. Carlson, F. R. Miller and A. B. Luckhardt for their suggestions and criticism throughout this work.

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THE MECHANISM OF THE RESPIRATORY WAVES IN SYSTEMIC ARTERIAL BLOOD PRESSURE

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To review the literature on the pulmonary circulation in its entirety is unnecessary. Tigerstedt (1903) and Wiggers (1921) have done this. Despite the efforts that have been expended in the study one is not left with a comprehensive conception of the manner in which mechanical changes in the thorax and pulmonary vessels accompanying respiration actually modify the pulmonary and systemic circulations.

That the venous return to the right heart is augmented during inspiration and decreased during expiration seems accepted by all. Experimental proof of this has been provided by Haller (1879), de Jager (1879), Burton-Opitz (1902), Hooker (1914) (1916) and Wiggers (1921). The chief factors responsible are the lowered intrathoracic pressure and the increased intra-abdominal tension. The blood is sucked into the thorax as it is pressed out of the abdomen. The output of the right ventricle is thus increased in inspiration, decreased in expiration.

The pressure changes in the pulmonary artery accompanying the respiratory act have been determined by Wiggers (1921) and by Tigerstedt (1903). The systolic and diastolic blood pressures in the pulmonary artery fall during the process of inflation of the lungs and rise during the process of deflation. With positive pressure ventilation of the lungs the opposite changes occur.

An attempt to discover the character of the respiratory waves in systemic blood pressure through the literature failed to reveal any uniformity in the findings reported. The reader is referred to Lewis (1908) for a tabulation of the earlier results. Recently Visscher, Rupp and Scott (1924) reported conclusions obtained through animal experimentation at variance with those found by Erlanger and Festerling (1912) in man. Unconvinced of any anatomical or physiological basis for such a discrepancy, it was decided to reinvestigate this problem using a method permitting the simultaneous recording of systolic and diastolic blood pressure changes.

EXPERIMENTAL. *Part I.* Five men with no known physical abnormalities were studied. Two sphygmoscopes of the type described by Erlanger

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and Meek (1926) adapted to employ the principle of Marey were used to record the blood pressure changes. Each sphygmoscope was connected with a pressure cuff applied to the arm just above the elbow. The pressure cuffs were filled from pressure tanks set at the level desired for each arm so that after each release the pressure could be automatically returned to the previous level. The respiratory movements were registered through a pneumograph held by a tape around the chest and attached to the tambour. The subject, comfortably seated in a chair with his arms as far as the elbows resting on a table in front of him, was instructed not to move and at all times to keep the glottis open. His blood pressure was carefully taken and the pressures in the tanks set so as to register just below systolic level in one cuff, just below diastolic in the other. To investigate abdominal breathing the subject was placed on a table in the supine position with thorax strapped and pneumograph held on the abdomen. Records of the thoracic type of respiration were also made in this position to serve as a basis for comparison with those made in the sitting position. The records were made when the breathing was 1, normal; 2, normal followed by prolonged inspiration; 3, normal followed by prolonged expiration, variations in the degree of quickness of onset of inspiration and expiration being made; also of 4, short inspirations followed by long expirations and vice versa; 5, very slow and shallow inspiration and expiration, 6, diaphragmatic in character.

Results. In normal respiration there was, as Erlanger and Festerling found, invariably an immediate diminution in the amplitude of the subsystolic pulse wave on inspiration, an immediate increase in amplitude on expiration. The subdiastolic pulse wave showed an immediate increase in amplitude on inspiration, an immediate decrease in amplitude on expiration (see fig. 1). When the durations of inspiration and expiration were within the normal limits these changes lasted throughout the particular phase of the respiratory act. When, after normal respiration, inspiration was prolonged the immediate diminution in amplitude of the subsystolic tracing lasting for 2 to 3 beats was followed by an increase in amplitude, then a decrease in the manner described as Traube-Hering waves (see fig. 2). The subdiastolic tracings showed pressure changes paralleling the above. When after normal respiration expiration was prolonged the immediate increase in amplitude of the subsystolic tracings for 4 to 5 beats was followed by a decrease in amplitude, then an increase in the manner described as Traube-Hering waves (see fig. 3). The changes in pressure as indicated by the subdiastolic tracings again paralleled the subsystolic ones. If very short shallow or long slow inspirations and expirations were taken (fig. 4) it was possible to practically eliminate all respiratory waves of blood pressure so that only waves described as Traube-Hering were seen. The changes occurring with the abdominal type of

respiration were similar to those occurring with the thoracic type. In all tracings the degree of change in amplitude seemed to vary directly with the force and depth of the respiratory phases. The rapidity of changes in amplitude seemed to go hand in hand with the rapidity of change in the respiratory phases. Special care was taken to note the possible existence of a lag in blood pressure changes but at all times no matter how rapid the onset of inspiration and expiration the alterations were immediate with the changes in the phase of respiration.

Interpretation of results. Through the work of Erlanger (1916) it has been shown that if by the principle of Marey a pressure lying anywhere between systolic and that giving maximum oscillations be applied to an artery an oscillation is recorded whose amplitude is intermediate between the two. If while this compression is maintained the arterial pressure should rise the amplitude of the recorded waves increases, if the arterial pressure should fall the amplitude of the recorded waves decreases. Hence, in our tracings an increase in the amplitude of the waves recorded under subsystolic compression indicates a rise in systolic blood pressure, a decrease in amplitude, a decrease in systolic blood pressure. Likewise when the pressure applied is subdiastolic an increase in amplitude of the recorded waves will indicate a decrease in diastolic blood pressure, a decrease in amplitude an increase in diastolic blood pressure provided the systolic blood pressure has remained constant. If this likewise varies the subdiastolic tracing is the resultant of changes in systolic and diastolic pressures. In any event a simultaneous increase in the amplitude of the subsystolic oscillations and decrease in the amplitude of subdiastolic oscillations indicate a rise in arterial pressure, the opposite combination a fall.

Consequently the result of the analysis of the tracings secured shows that there is an absolute drop in peripheral blood pressure during normal inspiration, an absolute rise during normal expiration. The initiation of each change is immediate with the change in the phase of the respiratory act. If respiration is prolonged the immediate fall of blood pressure present during the beginning of inspiration is followed by a rise, and the rise at the beginning of expiration is followed by a fall. So-called abdominal respiration gives a picture exactly similar to the thoracic types. The respiratory waves of blood pressure can be practically eliminated by very slow or very shallow breathing.

Theoretical considerations. To have a fall of systemic arterial blood pressure during inspiration when there is known to be an increased venous return to the right heart and consequently an increased volume output to the lungs one must assume either a diminution of volume output of the left heart or a decrease in peripheral resistance during this phase of respiration. There is no evidence to indicate that there is a decrease in periph-

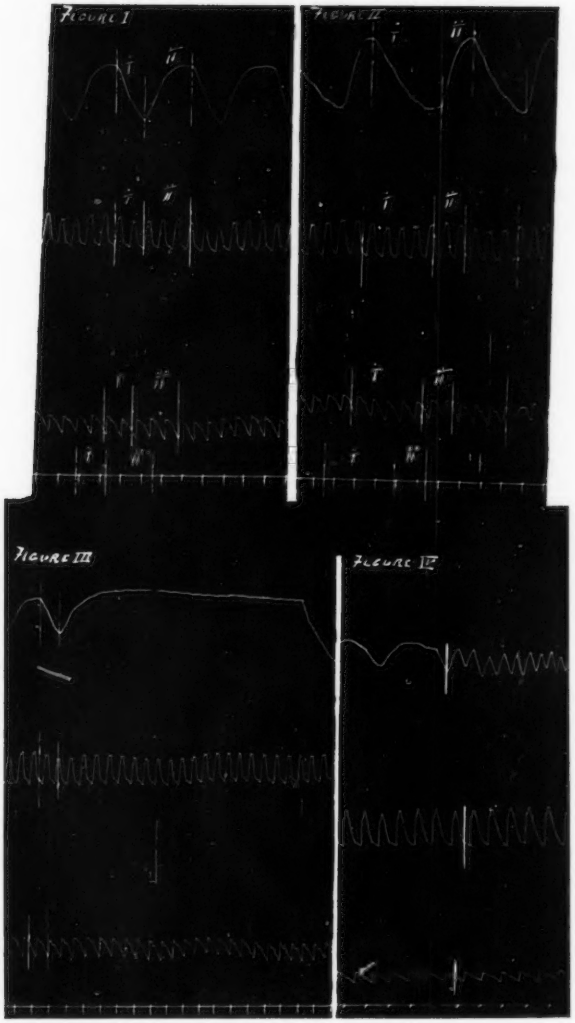

Fig. 1. Normal respiration. Upper curve, respiratory movements, down-stroke indicating inspiration, up-stroke expiration. Middle curve, subsystolic tracing. Lower curve, subdiastolic tracing. Time in seconds. Measurements made between the highest and the lowest point on each pulse wave.

Fig. 2. Prolonged inspiration showing the rise of blood pressure following the usual fall. Order of tracings similar to those of figure 1.

Fig. 3. Expiration prolonged. Note Traube-Hering waves. Order of tracings similar to figure 1.

Fig. 4. Quick shallow respirations showing elimination of blood pressure waves. Order of tracings similar to figure 1.

eral resistance, consequently one is forced to accept a lessened volume output. This again would indicate a diminished outflow from the lungs to the heart. With the outflow from the lungs decreased at a time when the inflow is increased there must be an increase in the capacity of the lung bed. In expiration exactly the opposite conditions are present. The rise in peripheral blood pressure must be due to increased output from the lungs to the heart. At the same time we know the inflow into the lungs is decreased. Consequently, during expiration the lungs contain less blood than during expiration. Inspiration must facilitate the flow of blood into the lungs, expiration its outflow.

EXPERIMENTAL. *Part II.* To study experimentally the changes in blood flow and vascular bed capacity occurring in the lungs during respiration the perfusion method was employed. With it all variables except those resident in the lungs themselves are eliminated.

Fresh lungs of the sheep, dog and cat were studied. They were inflated by negative and positive pressure. The perfusing fluid used was either defibrinated blood or this mixed with normal saline. The lungs were perfused at an approximately constant pressure. Changes in the rate and in the amount of inflow and outflow were registered by means of volume recorders. Inasmuch as many experiments were performed with invariably the same results only a type experiment with the records obtained will be described.

Technique. Two large cats were employed because the amount of perfusing fluid obtainable from one is insufficient. The cats were anesthetized with 20 per cent urethane, 7 cc. per kilo being administered through a stomach tube. They were then bled to death from the carotid artery. In order to thoroughly wash out the lungs normal saline was given intravenously toward the end of the bleeding process. The blood was defibrinated, diluted with normal saline and used as a perfusing fluid. The lungs from one of the cats were then removed. Cannulas were inserted into the trachea, the pulmonary artery and the left auricle. The latter was tied in a mass ligature about the cannula so that its mouth rested at the openings of the pulmonary veins in the auricular cavity. The lungs were then placed in the bell jar shown in the diagram (fig. 9). Negative pressure was produced by means of a water suction pump. A filtering flask placed in the suction line permitted the rapid inflation of the lung. Deflation was brought about by diminishing the negative pressure in the bell jar through a three-way stopcock opened to the air. By this means it was found easy to obtain all stages and degrees of inflation and deflation and also to vary the rapidity of occurrence of these processes. The perfusing fluid was placed in a Marriotte flask 100 cm. above the level of the lungs. A pressure bottle of about 200 cc. capacity was placed in the inlet line 40 cm. above the level of the lung. A similar pressure bottle in the out-

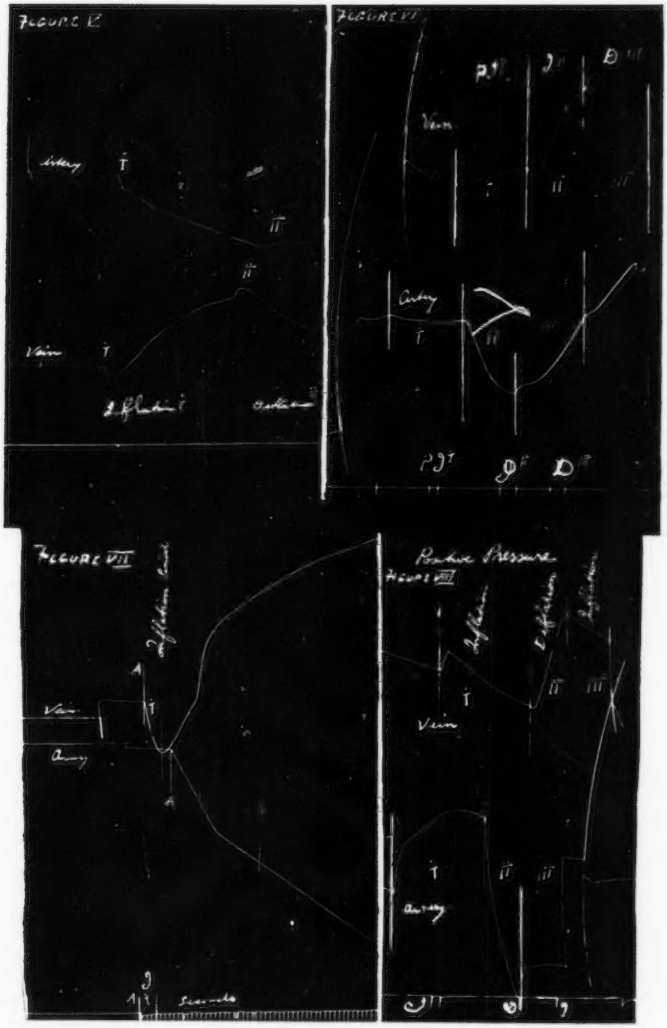

Fig. 5. Typical tracing obtained in lung perfusion experiment. Lung expanded by negative pressure. Up-stroke in the arterial tracing here as in figures 6, 7 and 8 indicates a diminution in inflow, in the venous tracing an increase in outflow. Down-stroke in the arterial tracing indicates an increase in outflow, in the venous tracing, a diminution in outflow.

Fig. 6. Tracing obtained in lung perfusion experiment. Lung expanded by negative pressure. *PI* represents partial inflation, *I*, complete inflation, *D*, deflation.

Fig. 7. Tracing obtained in lung perfusion experiment. Lung inflated with negative pressure. Time marked in seconds. Note the drop in the inflow recorder extends over the exact time interval during which the lungs are being expanded, also that the blood flow changes occur immediately on changing the state of inflation of the lungs.

Fig. 8. Tracing obtained in lung perfusion experiment. Lung inflated with positive pressure. Note that the changes in direction of the recording levers during inspiration and expiration are exactly opposite to those of figure 5.

let line rested 5 cm. below the level of the lung. The inflow into the first pressure bottle and the outflow from the second pressure bottle were regulated by means of screw clamps. These clamps were so adjusted that the writing points connected with the pistons on the volume recorders ran parallel to each other and the base of the drum. Then the lungs were inflated and deflated as desired. When positive pressure inflation was used this was obtained by blowing into the tube attached to the trachea. It is most essential to appreciate that the inflow into the first capacity bottle from the Marriotte flask is constant and that the outflow varies with the lung inflow, these being the changes recorded by the writing point. In our experiments all stages of inflation and deflation with both negative and positive pressure were employed.

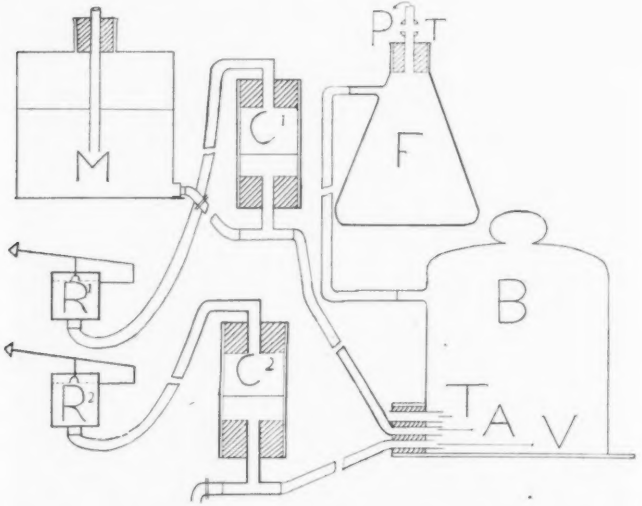

Fig. 9. Diagram of apparatus for lung perfusion. *B* bell jar with *T*, *A V*, representing the connections for the tracheal, arterial and venous cannulae. *F*, filtration flask with three-way stopcock, *T*, and suction pump connection, *P*. *M*, Marriotte flask connected to pressure bottle, C_1, the latter then connected to the arterial cannula. A screw clamp on this line regulates the flow to pressure bottle, C_1. C_2, pressure bottle connected with venous outflow, outflow regulated by screw clamp. R_1 and R_2 volume recorders connected with C_1 and C_2.

Results. When the lungs were inflated by negative-pressure (figs. 5, 6 and 7) the piston recording the inflow fell suddenly and rapidly throughout the period of inflation and continued to fall at a constant rate throughout the time the lung was in the inflated state. The piston recording the outflow fell during the actual period of inflation and then rose at a constant

rate throughout the time that the lungs were in the inflated condition. On deflation the inflow recording piston rose immediately and rapidly and continued to rise throughout the deflated state. The outflow recording piston rose during the actual period of deflation and then during the deflated state fell at a constant rate. When positive pressure was used to inflate the lungs (fig. 8) the opposite results were obtained.

Interpretation of results. An analysis of the above tracings shows without the slightest doubt that when negative pressure is employed there is during the actual period of inflation an increased flow into the lungs with a diminution in the outflow. After inflation has been completed the outflow increases at a rate corresponding to the continued but now constantly increased inflow. The inflated lung accommodates more blood than the deflated lung and offer less resistance to the flow of blood. During the process of deflation there is a constantly diminishing inflow, but a constant and relatively slow rate during the deflated state. The outflow, on the other hand, increases during the period of deflation and then diminishes to a constant rate corresponding to the diminished inflow. The deflated lung accommodates less blood and offers more resistance to blood flow than the inflated lung.

With positive pressure the opposite conditions hold. On inflation of the lungs there is a diminution of the rate of inflow to a slower constant rate, and an increase followed by a decrease to a constant in the rate of outflow. When the lungs are allowed to collapse the rate of inflow increases to a constant level, while the outflow first decreases and then increases to a constant level. The expanded lungs have a smaller vascular capacity than the deflated lung. It is particularly important to note that changes in inflow and outflow occurred immediately on changing the volume of the lung.

CORRELATION OF THESE RESULTS WITH THOSE THEORETICALLY ESSENTIAL TO EXPLAIN THE CHANGES OCCURRING IN PERIPHERAL BLOOD PRESSURE DURING RESPIRATION. It will be readily seen that the results obtained experimentally are exactly those theoretically necessary to explain both the immediate fall in blood pressure on inspiration and the immediate rise on expiration. They explain, too, the rise in blood pressure which follows the first fall in prolonged inspiration. The fall occurs during the period of inflation when the increase of capacity of the pulmonary bed slows the outflow. The subsequent rise is the expression of the increased outflow consequent to the increased inflow and reduced resistance. It takes some time for the increased inflow to make itself felt upon the outflow side. The rise in blood pressure immediately on expiration is the result of an increased outflow due to a diminution in the size of the vascular field forcing out the blood it contains. The subsequent fall occurs because of

the diminished inflow caused by increased resistance when the lungs are deflated.

Lewis (1908) and others have shown that respiratory waves in blood pressure are opposite to those obtained by us during normal respiration when positive ventilation is employed. We have observed the same in dogs both with the chest open and the chest closed. These findings are readily explained through the results obtained in our experiments when positive pressure is employed for inflation.

In our experiments with man we found that the fall in peripheral blood pressure occurring at the beginning of inspiration lasts throughout the period of inspiration when this is of normal length. Likewise the rise in blood pressure seen in expiration normally lasts throughout that period. In our experiments we found that the initial changes are spread over a sufficient time to account for these findings. Normally the time required to inflate the lungs is about two heart beats, to deflate them about three heart beats. We have found that when inflation and deflation are carried out slowly the initial diminution in outflow present on inflation is slight or absent, as is the case with the initial rise on deflation. Above we noted the fact that respiratory waves in blood pressure can be made very slight or eliminated by very slow breathing. This is also the case with short shallow respirations. All our tracings showed that the degree of initial changes varied directly with the rate of inflation or deflation of the lungs.

Earlier we noted the changes in blood pressure occurring in the pulmonary artery with respiration. The fall in blood pressure on inspiration during the period of inflation now is seen to be due to the fact that the diminished resistance and the increased capacity of pulmonary vascular bed first more than compensate for the increased output from the right ventricle. The subsequent rise in blood pressure follows when the pulmonary bed has been filled. The reversal of these findings on positive ventilation is what one would expect. In all of our experiments it was noted that a slight change in the pressure within the bell jar had a pronounced effect on the blood flow through the lungs. This is due to the relatively low blood pressure existent in the lung capillaries.

Discussion. Previous experimenters, especially de Jager (1879), and Quincke and Pfeiffer (1871), have perfused lungs with results very similar to our own. Their methods in recording the changes in inflow and outflow were different and we believe their interpretation of the facts was incorrect.

Quincke and Pfeiffer found an increased capacity of the vascular bed of the lungs in inspiration in comparison with expiration. They also found the opposite conditions to hold in lungs inflated with positive pressure. However, they considered the change in capacity of the heart and of the blood vessels of the thorax, resultant from the increased negative pressure during the inspiratory phase, more than counterbalanced the increased capacity in the pulmonary bed. Hence according to them the flow

through the lungs is diminished in inspiration. This we now know is incorrect. D'Arsonval (1877) perfused lungs with defibrinated blood which was allowed to enter through the inferior vena cava below the diaphragm and was collected from the abdominal aorta. The phases of respiration were produced by traction on and relaxation of the diaphragm. There was first a lessened outflow until the lungs were inflated and then an increased outflow.

De Jager after very extensive and careful investigation felt convinced that the pulmonary bed was increased during inspiration but argued that the flow through the lungs was lessened in inspiration in intact animals because when the arterial inflow and venous outflow pressures were varied by the same inflation pressure as was applied to the lung the inflow is diminished. This he believed represented the normal condition in the chest. However, we know that the negative pressure exerted on the vessels in the thorax acts through the vessel walls and not directly on the driving pressure of the blood. The effect through the veins is decidedly greater than it is through the arteries. In our experiments the same negative pressure was applied to the arteries and veins and it has been seen that under these conditions, representing the true normal, the flow during inspiration is increased.

Tigerstedt, because he was able to tie off large portions of lung without any significant change in the peripheral blood pressure, concluded that changes in pulmonary resistance were of such slight consequence as to be negligible as factors in the production of respiratory waves in blood pressure. Such an assumption disregarded the fact that he was working with animals with a very movable mediastinum so that the unaffected portions of the lung could easily expand. Then it is also well known that under such circumstances new capillaries open up to accommodate the altered circulation. He concluded that change in blood flow to the thorax and consequently the right heart during inspiration was the chief factor in the production of respiratory waves in blood pressure. He apparently felt his position strengthened because he thought respiratory waves of blood pressure in some animals, mentioning the dog, differed from those in man and other animals. That this is not the case has been demonstrated by Lewis (1908); and other investigators have found the dog to be similar to man in that there is a fall of blood pressure during inspiration, a rise during expiration. We have confirmed these observations in the dog. The fact that respiratory waves in blood pressure, opposite in sign, to be sure, to those obtaining usually, occur during positive pressure ventilation with the thorax open when changes in blood flow to the right heart are at a minimum practically rules this out as the causal factor in the production of these waves. The increased flow of blood to the right heart during inspiration simply works hand in hand with the increased capacity of the

vascular bed of the lungs during this phase of the respiratory act. We believe that the only factors truly causative in the production of respiratory waves in blood pressure are the changes in vascular capacity and resistance in the pulmonary bed accompanying inflation and deflation of the lungs. To have the pulmonary vascular capacity greatest during inspiration when a fresh supply of air is available for oxygenation of the blood is a condition much more advantageous to the organism than its opposite would be.

Cloetta (1912), the outstanding exponent of the view that during the height of inspiration blood flow through the lung is impeded, bases his conclusions on erroneous experimental findings. His tracings show either no change or a rise in pulmonary arterial pressure during inspiration. This we know is incorrect. Undoubtedly, during the normal inspiration the pulmonary arterial pressure falls. Had he obtained the correct finding using his own reasoning one would have had to grant an increased flow through the lungs during inspiration. On the basis of microscopic sections he found the collapsed lungs to contain more blood than the inflated lungs. The conditions under which he secured the lungs for making sections were entirely abnormal and examination of his published sections reveals that the alveoli in the collapsed lung have an area of only about one-third of those of the expanded lung. This would indicate a degree of collapse far greater than the normal and nullify entirely the value of any comparison.

SUMMARY

Experimental evidence is produced to show that pulmonary resistance is diminished and vascular capacity increased during inflation of the lungs. On deflation of the lungs pulmonary resistance is increased while the vascular capacity is diminished. The opposite conditions hold in lungs inflated by positive pressure.

By an improved method it is shown that in man the respiratory waves in systemic arterial blood pressure consist of an immediate fall on inspiration, an immediate rise on expiration.

These facts are adequately explained on the basis of passive changes occurring in the capacity of the vascular bed of the lungs during the respiratory phases.

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A COMPARISON OF THE BLOOD PRESSURE, KIDNEY VOLUME AND THE PANCREATIC SECRETORY RESPONSE FOLLOWING THE VEIN ADMINISTRATION OF VARIOUS SECRETIN PREPARATIONS

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Since the discovery by Bayliss and Starling (1902) of the presence in the intestinal mucosa of an extractable substance, pro-secretin, a number of articles have been published (Popielski, 1903; Dale and Laidlaw, 1912; Matsuo, 1912–13; Skarzynska, 1924; Luckhardt et al., 1926), showing the secretory response of the pancreas to secretins made by various methods.

The undoubted pancreatic secretory response elicited by the vein administration of secretin has been ascribed to the vasodilatin content of the extracts (Popielski, 1903) and to a specific action of the mucosal extracts on the pancreatic secretory mechanism irrespective of the vasodilatins (Carlson et al., 1916; Luckhardt and Blonder, 1924; and Parsons, 1925). The proof of the latter contention has recently been shown for pure pancreatic secretin by the preparation of a highly effective vasodilatin-free secretin (Weaver et al., 1926).

No one, however, has attempted to analyze carefully the specific effects of the vasodilatin and the vasodilatin-free fractions of the standard secretin preparations in order to show either a possible antagonism or synergism of the two constituents of the mucosal extracts in relation to pancreatic secretion.

The present investigation was undertaken as an attempt to study the relationship of various secretagogue preparations, i.e., HCl in the gut, and the following secretins, Bayliss and Starling; Dale and Laidlaw, "new" secretin, and the vasodilatin-free secretin of weaver et al.—as to their relative secretory efficiency under standard conditions and to note the general and local vascular changes accompanying the increased secretion, both before and after atropinization and before and after cutting the vagi, with the idea that such a correlation by one worker would give more comparable figures for all the secretagogues and at the same time more clearly demonstrate the isolated observations of various authors.

METHODS. During the course of study 28 dogs, ranging in weight from 15 to 20 kilograms, were used. Eighteen to 24 hours after feeding, the

animals were placed under a light ether anesthesia (as a preliminary to the administration of the hypnotic). The femoral vein was then exposed and a cannula inserted and connected with a burette for washing in injections. The ether mask was then removed and barbital sodium in a dosage of 225 mgm. per kilo body weight slowly injected.

Blood pressure was taken from the carotid artery and recorded by a mercury manometer.

The kidney volume was recorded by means of an egg-shaped oncometer which was made in two halves hinged together, the inner surface of each half being covered with rubber dam. The kidney after being inclosed in the oncometer was replaced in the abdominal cavity. The volume changes were recorded by air transmission by connecting the two oncometer lead off tubes to a recording tambour. In a few of the initial experiments the vascular changes of the pancreas were followed but the difficulty of obtaining records over a long period were found to be too great since the pressure necessary to make a recordable tracing soon produced a hemorrhagic gland and at the same time interfered with the collection of juice. As a consequence, since the kidney gave tracings identical with those obtained from the pancreas, the kidney volume changes are interpreted as an index of the volume changes of the splanchnic organs, including the pancreas.

The stomach was then isolated and a ligature tied securely at the pyloric valve.

In those experiments requiring the injection of 0.2 per cent HCl into the intestine, the latter was isolated 10 to 12 inches below the pancreas and an intestinal cannula placed in the gut wall. Access to the intestinal lumen was by means of a rubber tube extension of the cannula extending through the abdominal incision.

The pancreas was isolated and the duct of Santorini cannulated and a short rubber tube added to the cannula for leading the juice through the incision, after which the duodenum was replaced in the abdomen and all incisions closed by sutures.

The procedure of an experiment was to take a simultaneous graphic record of the blood pressure, kidney volume and pancreatic secretion over a ten-minute period, preceding any injection. After obtaining constant values for these observations, an injection of Bayliss and Starling secretin, in a dosage representing 0.034 gram of the original mucosa per kilo body weight, was made. At the end of 30 minutes, by which time the blood pressure and pancreatic secretory rate had returned to normal, a dosage of the second secretagogue as closely corresponding, in terms of intestinal mucosa, to that of the standard secretin, i.e., Bayliss and Starling, was injected. These procedures were alternated during the succeeding 9 to 12 hours on each of a series of 5 to 7 dogs for each secretagogue.

RESULTS. *HCl in intestine.* The general vascular response following the injection of 100 cc. of 0.2 per cent HCl into the intestinal lumen is in contrast to that obtained from the control injection of Bayliss and Starling secretin (fig. 1). Instead of a marked fall in blood pressure a rise was observed, the degree of which might be explained as due partly to an increased abdominal pressure and partly from reflexes from the local stimulation.

The splanchnic vascular change observed, i.e., kidney volume, as a result of the HCl injection was a definite vasodilatation which persisted throughout the period of accelerated pancreatic secretory activity. This observation confirms those of Burton-Opitz that the introduction of 10 to 15 cc. of 0.4 per cent HCl in the duodenum causes an increased blood flow to the pancreas and duodenum of over 100 per cent per second. The splanchnic response to the vein injection of Bayliss and Starling secretin undoubtedly was comparable to that obtained with the HCl. The initial drop in general blood pressure would so diminish the blood supply to the kidney that any initial vasodilatation would be masked, as shown by the recorded vasodilatation of the kidney five minutes before the complete recovery of the general blood pressure.

The secretory response of the pancreas to the injection of HCl into the duodenum confirms the observations of Dolinski (1894), Popielski, Enriques and Hallion (1903), Pawlow (1912) and others in that a copious secretion results, beginning after a 2-minute latent period and continuing during the succeeding 40 minutes.

Dale and Laidlaw secretin. This preparation in the hands of the author was not vasodilatin-free, although the vasodilatin content was smaller than that present in the control, i.e., Bayliss and Starling secretin (fig. 2).

A definite splanchnic vasodilatation was evident after the 5th minute with both secretin preparations. The latent period and the maximal secretion peak were identical for the two preparations although the removal of a portion of the vasodilatins from the Dale and Laidlaw secretin markedly impaired its secretory efficiency.

New secretin and vasodilatin-free secretin. The new secretin was prepared by the method of Luckhardt, Barlow and Weaver (1925) in a concentration approximately one-half that of the control, Bayliss and Starling secretin, i.e., a dog of 15 to 18 kilograms body weight will yield approximately 25 grams of fresh mucosa from the upper four feet of the intestine. The mucosa worked up by the Bayliss and Starling procedure would yield 100 cc. of extract, and by the method of Luckhardt et al., 200 cc. of extract. The vasodilatin-free secretin was made by dividing the total volume of a new secretin preparation in half, using the first half as a control and purifying the second half and bringing up to volume.

The relative vasodilatin content of the three secretins is well shown

in figure 3. The new secretin contains a small amount of vasodilatin giving a maximal drop in blood pressure of 8 per cent, while the purified preparation either does not affect the general blood pressure or gives a small pressor reaction. Both the new secretin and the purified new secretin result in an increased organ volume. This response was duplicated with Bayliss and Starling secretin after the recovery of the general blood pressure to 80 per cent normal.

The secretory responses to the three secretin preparations are parallel in latent period, time of maximal secretion and direction. There is a marked difference, however, in their relative secretagogue efficiencies. By comparing the blood pressure and organ volume changes of curves *A* and *C*, figure 3, it seems evident that the secretory response to secretin is due to two factors, i.e., pancreatic secretin, and within a reasonable range, possibly the vasodilatins as well, particularly since the secretion curve has a value inverse to the blood pressure values. The proof of the vasodilatin secretagogue action has been shown by Popielski and confirmed by the author. These observations do not confirm the work of Skarzynska (1924) who prepared two fractions from intestinal mucosal extracts, one of which gave pure secretory responses with no vascular change, while the other gave purely vasodilatin but no secretagogue response. However, the observation of Weaver, Luckhardt and Koch (1926) that the presence of vasodilatin seems to hinder the efficiency of the secretagogue certainly holds true when the blood pressure falls to and remains near the critical level for a prolonged period. The difference in the secretagogue efficiencies of *A* and *B* might thus be due to the presence in the former of more vasodilatins as a result of the method of preparation, rather than an incomplete extraction of the true secretin by simply placing acid in the intestine for a 30-minute period.

Removal of vasodilatins from Bayliss and Starling secretin. The vasodilatins may be partially removed from Bayliss and Starling secretin by repeatedly washing the latter with saturated NaCl in 0.4 per cent HCl (fig. 4). The secretagogue efficiency, however, decreases parallel with the degree of purification or vasodilatin removal. The loss of the secretin fraction during the purification process seems closely related to removal of the proteins since the sterilization of practically vasodilatin free preparations of new secretin by boiling increased the vasodilatin fraction (Weaver, Luckhardt and Koch, 1926).

Effect of atropine and cutting the cervical vagi. The external secretory response of the pancreas to secretin preparations was not influenced by the administration of $\frac{1}{4}$ mgm. of atropin at 30-minute intervals. The responses before and after atropin were of the same order of magnitude as previously determined by Farrell and Ivy (1926), and Bayliss and Starling, (1902) although the rate after atropin was slightly higher.

The response of the pancreas to a standard dose of secretin before and after cutting the cervical vagi did not materially differ. The secretion after cutting the vagi was somewhat less in volume but this difference might as easily be explained by the resulting lower blood pressure as well as the poorer vascular recoveries made by the animals after secretin injections, rather than removal of central secretory impulses (Popielski, 1903; Pawlow, 1912).

SUMMARY. A comparison of the secretins of Bayliss and Starling, Dale and Laidlaw, Luckhardt et al. and Weaver et al., with the normal pancreatic secretion stimulus, i.e., acid chyme, (Pawlow), or acid in the duodenum, shows the following similarities: the splanchnic organs are dilated with a resultant increase in blood flow to the pancreas, duodenum (Burton-Opitz, 1920) and kidney volume, and an apparently purely local stimulating effect on the pancreas, i.e., independent of central secretory impulses, since neither atropin in very large doses nor cutting the cervical vagi influenced the secretory responses. The similarity between the vascular and secretory responses obtained from the several secretin preparations and the accepted physiological reaction following the introduction of dilute HCl, comparable to the expulsion of acid chyme into the small intestine, seems too great to be merely accidental, the greatest difference among the secretins observed being due to their variability in the relative vasodilatin and secretin fractions.

Bayliss and Starling secretin on injection produces a double reaction, 1st, a marked general splanchnic vasodilatation, and 2nd, pure stimulation of the pancreatic secretory mechanism; both actions being supplementary, i.e., the vasodilatins actually increase the secretory response (fig. 3, curves *B* and *C*, and fig. 4, curves *A* and *B*).

CONCLUSIONS

1. A comparison of the general and splanchnic vascular changes as well as the pancreatic secretagogue efficiency following the injection of three vasodilatin-containing secretins and one vasodilatin-free secretin has been made.

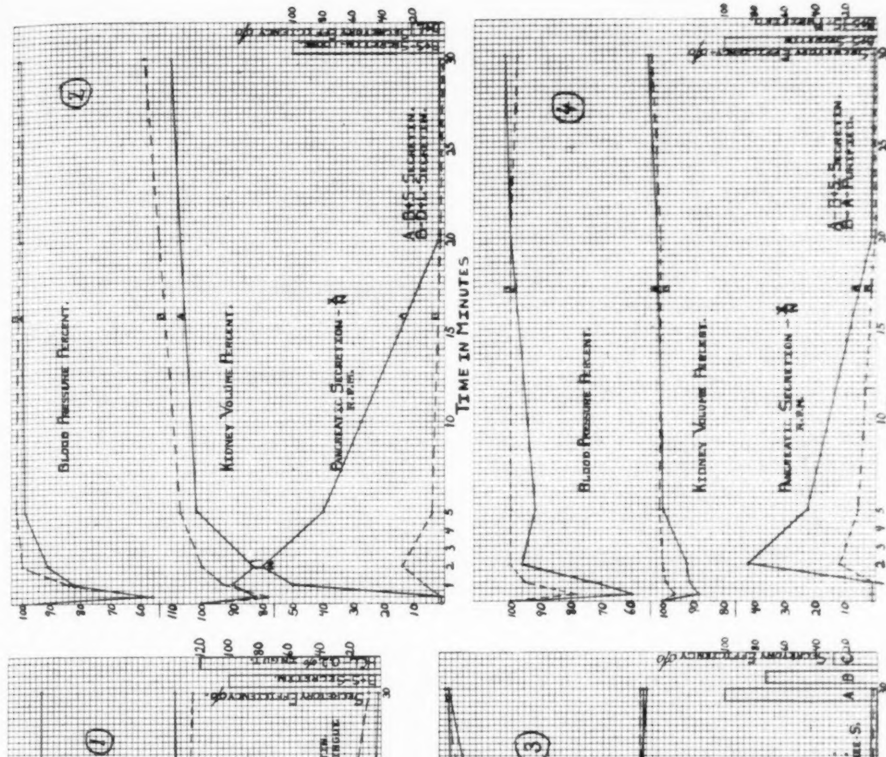
Figs. 1, 2, 3 and 4.

The blood pressure has been computed on the basis of 100 per cent, the pressure in millimeters Hg immediately preceding the injection being considered 100 per cent.

The kidney volume changes were computed in per cent by arbitrarily considering 1 mm. change in the graphic record equivalent to 1 per cent.

The secretory efficiency was computed by deriving the factor

$$\frac{X = \text{the rate in drops at any minute following the injection}}{N = \text{normal secretion rate in drops before injection}}$$



Blood Pressure Percent.
Kidney Volume Percent.
Pancreatic Secretion
Time in Minutes

2. The similarity between the responses to several secretins and those observed during normal physiological processes are discussed.

3. The secretagogue efficiency of the vasodilatin and the secretin fractions of standard secretin preparations are discussed.

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THE INFLUENCE OF SODIUM NITRITE, PEPTONE AND PILOCARPINE ON THE EXTERNAL SECRETION OF THE PANCREAS

A Correlation of the General and Splanchnic Vascular Changes with the Increased Pancreatic Secretion Rate

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In an earlier publication, in which the vascular and pancreatic secretory changes following the vein administration of standard secretin preparations were correlated (Barlow, 1926) it was observed that a unit dose of a control secretin, containing a small amount of vasodilatins, showed a higher secretagogue efficiency than an equivalent dose of a vasodilatin-free secretin (Weaver et al., 1926) made from a portion of the control extract.

The present study was undertaken in an attempt to clarify the above assumption, i.e., to determine whether vasodilatins per se may actually be additive with pancreatic secretins and whether they may act as pancreatic secretagogues as postulated by Popielski (1912).

Since the vasodilatin principles as represented by certain portions of intestinal mucosal extracts are of relatively unknown character, it was deemed advisable to repeat the work of Popielski (1912) with Witte's peptone and to duplicate the results through the use of a known chemically pure vasodilatin substance, i.e., sodium nitrite. The responses obtained from peptone and sodium nitrite were then compared with two other types of pancreatic secretagogues, i.e., Bayliss and Starling secretin and pilocarpine respectively, with the idea of interpreting the mode of action of the vasodilatins. The usual pancreatic secretory responses obtained with them were then compared with those obtainable after cutting the cervical vagi and after atropine and ergotamine tartrate respectively.

Methods. During the course of study, 36 dogs ranging from 15 to 20 kilograms in weight were used. Eighteen to 24 hours after feeding, the animals were placed under a light ether anesthesia, preliminary to the administration of the general hypnotic. The femoral vein was quickly isolated, and a cannula inserted and connected with a burette for washing in injections. The ether mask was then removed and barbital sodium, in a dosage of 225 mgm. per kilo body weight, slowly injected. The

animals were thus maintained under very constant conditions as to anesthesia, blood pressure and respiration rate during the succeeding 10 to 12 hours of the experiments.

General blood pressure was recorded from the carotid artery by the usual methods. The splanchnic vascular changes were recorded from the kidney volume by means of an egg-shaped oncometer, each inner half of which was fitted with a rubber dam, the graphic record of the volume changes being obtained by air transmission from the oncometer to a tambour.

The pyloric valve was tightly ligated in all except a few experiments. In the latter a loose ligature was placed around the pylorus and the ligature ends led through the abdominal wall by means of a metal tube similar to that frequently used for constricting arteries. In this manner the pylorus could be ligated or freed at will simply by varying the tension on the ligature ends, with the minimal disturbance to the animal. The duodenum was then isolated and a small glass cannula inserted in the duct of Santorini and ligated in place, the pancreatic juice being led to the exterior by means of a short rubber tube attached to the cannula. The duodenum was then replaced in the abdomen and all incisions closed by sutures.

Results. In order to have some standard criteria by which to judge the secretagogue efficiency and the vascular changes produced by the preparations to be tested, a standard dose of Bayliss and Starling (1902) secretin, representing 0.034 gram of the original intestinal mucosa per kilo body weight, was used as a control, the same secretin preparation thus being compared with the respective secretagogues on each dog of a series.

The usual course of an experiment was to obtain a simultaneous graphic record of the blood pressure, kidney volume and pancreatic secretory rate during a ten-minute period. A secretin injection was then made and the observations continued until the blood pressure and secretory rate returned to that immediately preceding the injection. A dose of the vasodilatin was then injected and the observations repeated during the succeeding 40 minutes.

Sodium nitrite. The general and splanchnic vascular changes obtainable after the vein administration of 15 mgm. of sodium nitrite are shown and compared with those obtainable from a standard dose of secretin (fig. 1). The general blood pressure after nitrite shows the typical gradual fall to a maximum at the 5th minute with a gradual progressive recovery thereafter. The splanchnic vascular changes, including the pancreas, show a definite dilatation and in spite of the slight decrease in kidney volume after the 2nd minute, the splanchnic vasodilatation is even more evident when the blood pressure level is observed.

The increased pancreatic secretion following the administration of sodium nitrite shows a latent period and a maximal peak corresponding to

that of the control secretin injection. Likewise the nitrite secretory period corresponds to that observed for secretin in that the secretory values bear an inverse relation to the general blood pressure values. Consequently, since the blood pressure was depressed by sodium nitrite over a period longer than that of the secretin, the secretory values remain above normal correspondingly.

The external pancreatic secretory rate was increased with all dosages of sodium nitrite studied, i.e., 2 to 300 mgm. The secretory efficiency increased with the dosage up to a maximum which was obtained with an optimum dosage of 20 mgm. With larger doses the secretory efficiency decreased from the maximum, inverse in degree to the size of the dose administered.

Gottlieb (1894), in a study of the relations between the pancreatic blood supply and the secretion rate, concluded that secretion seemed dependent on the amount of blood coming to the gland and quotes Heidenhain to the effect that the connection between glandular activity and vessel width was unmistakable. It seems quite probable that the most important factor governing the external pancreatic secretion, following the administration of sodium nitrite is the total blood supply to the gland and this in turn directly dependent on the degree of splanchnic vasodilatation (Burton-Opitz, 1910; Sollmann, 1922) and the general blood pressure level. Low blood pressure in itself does not necessarily favor pancreatic secretion but dilatation of splanchnic vessels certainly favors an optimum secretion. Consequently with the larger nitrite dosages administered, the marked and prolonged fall of blood pressure tended to embarrass the general functions, including the pancreas, irrespective of the marked splanchnic dilatation as shown with different methods by Franck and Hallion (1896).

It is of interest to note in comparison with the nitrite reactions that frequently barium chloride has been observed to increase the pancreatic secretory rate through a vasodilatation of the pancreatic vessels (Edmunds, 1910–11).

The nitrite secretagogue action may be obtained following the injection of sodium nitrite either initially, i.e., the first injection in any dog, or the total secretion obtainable with a certain dosage may be duplicated in sequence over a period of three hours; further, the volume of juice obtained at the beginning of an experiment compares favorably with that obtainable under the same conditions twelve hours later.

The pancreatic responses obtainable, both preceding and following ligation of the pyloric valve, or the intravenous administration of large doses of atropine, or ergotamine tartrate, 1 mgm. per kilo body weight, and repeated at intervals, are of the same order of magnitude. In two out of twenty-nine injections in six different dogs, an optimal dose of sodium nitrite gave a greater secretory response preceding ligation of the pylorus

than was obtained afterwards. This response, obviously due to the expulsion of a small amount of acid from the stomach (Pawlow, 1912; Barlow, 1926) occurred near the end of the nitrite response and must be considered exceptional.

Synergism of pancreatic secretins and vasodilatins. The assumption that the vasodilatin and secretin fractions of standard secretin preparations are, within limits, additive, was tested out on 4 animals. The responses, obtainable in sequence, to a single dose of Bayliss and Starling secretin; 15 mgm. of sodium nitrite; to a dose of Bayliss and Starling secretin plus 15 mgm. of sodium nitrite, and to two doses of Bayliss and Starling secretin, were compared in figure 2.

The marked differences between the pancreatic secretory curve of the combined secretin and nitrite injection, curve *C*, and those of *A*, *B* or *D* might apparently be explained by the greater and more prolonged depression of the general blood pressure. The secretin contained two fractions, i.e., vasodilatin and pancreatic secretin (Weaver et al., 1926). Presumably the combined vasodilatation produced by the secretin vasodilatins and the sodium nitrite resulted in a secretion through purely vascular changes as well as furnishing the optimal pancreatic secretory conditions for the secretin fraction.

Peptone. The definite secretagogue reaction of the pancreas to the vein administration of Witte's peptone and to a lesser degree, Merck's peptone as well, figure 3, confirms the results of Popielski (1912) and Skarzynska (1924).

The general and splanchnic vascular responses to peptone are comparable with those obtained with sodium nitrite, the greatest difference being the degree of the peptone blood pressure depression. As a consequence, the pancreatic secretory response following the administration of Witte's peptone bears an inverse relationship to the blood pressure level during the first 20 minutes only, at which time the secretory rate has returned to normal while the blood pressure is only 80 per cent normal and the splanchnic vasodilatation maximal (Kondo, 1919).

The only explanation offered for these minor differences between the actions of the nitrites and peptones is an apparent difference in dosage and the complex character of the latter which, according to Hanke and Koessler (1920), consists mainly of proteoses, some peptone and various quantities

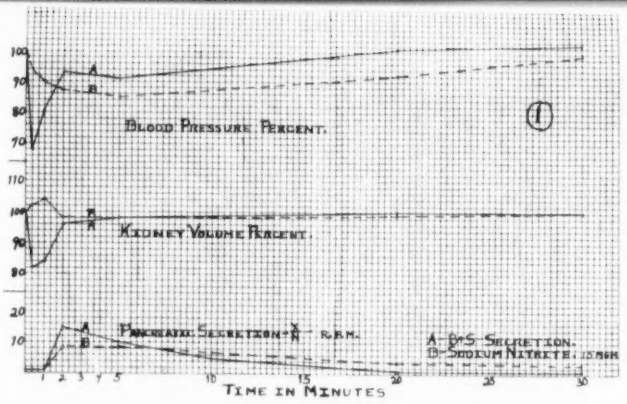
All graphs represent the median data for all dogs of each series.

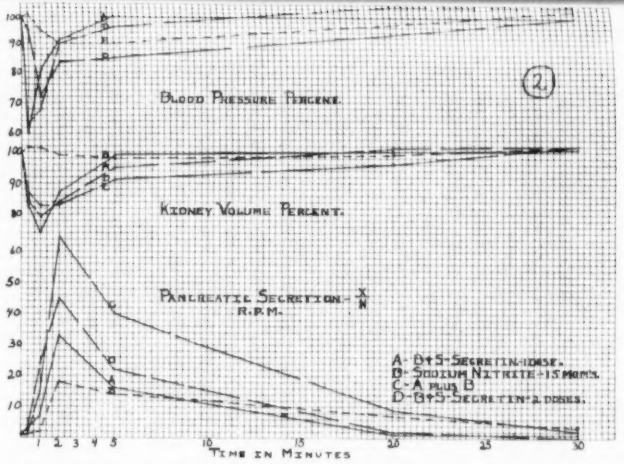
Figs. 1–4. Blood pressure computed in per cent of level preceding injection.

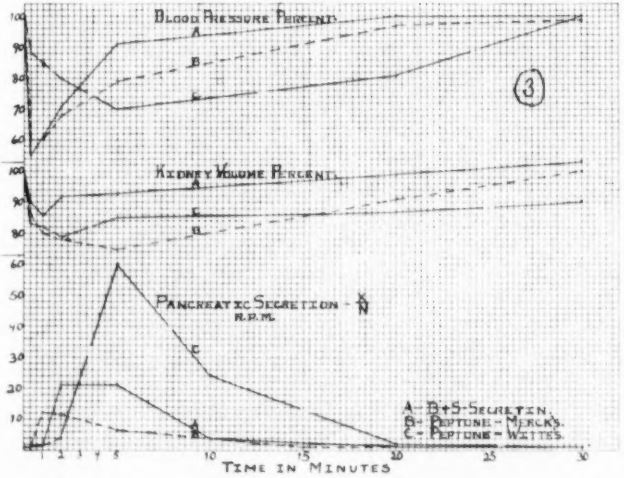
Kidney volume—a change of ± 1 mm. in the oncometric graphic record was arbitrarily taken as equivalent to ± 1 per cent.

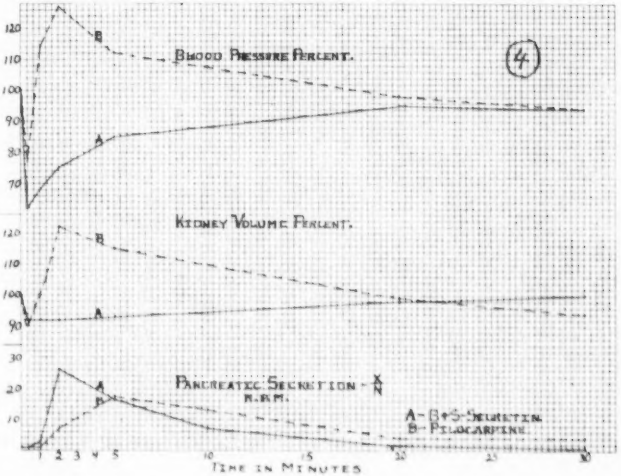
Pancreatic secretory rate—

$$\frac{\text{X} - \text{rate in drops per minute at a given period}}{\text{N} - \text{normal rate in drops per minute}}$$


1
Blood Pressure Percent.
Kidney Volume Percent.
Pancreatic Secretion - N/N R.P.M.
A- B+S Secretion.
B- Sodium Nitrite .15 Mgm.
Time in Minutes


2
Blood Pressure Percent.
Kidney Volume Percent.
Pancreatic Secretion - N/N R.P.M.
A- B+S-Secretin-Dose.
B- Sodium Nitrite .15 Mgms.
C- A plus B
D- B+S-Secretin-2 Doses.
Time in Minutes


3
Blood Pressure Percent.
Kidney Volume Percent.
Pancreatic Secretion - N/N R.P.M.
A- B+S-Secretin.
B- Peptone - Merck's.
C- Peptone - Witte's.
Time in Minutes


4
Blood Pressure Percent.
Kidney Volume Percent.
Pancreatic Secretion - N/N R.P.M.
A- B+S-Secretin.
B- Pilocarpine
Time in Minutes

of histamine. In this connection it may be observed that although the secretory efficiency of Merck's peptone was found to be much greater than similar data presented by Skarzynska (1924), the pancreatic secretory response to Merck's peptone, as observed by the author, compares favorably with similar data by Skarzynska (1924), Parsons (1925) and Gutkowski (1926) for histamine.

The peptone secretagogue efficiency follows a curve similar to that of sodium nitrite, in that the efficiency increases with the degree of vasodilatation up to a maximum obtained with a dosage of 30 cc. of a 5 per cent solution and decreases with higher dosages.

Pilocarpine. The comparison of the vascular and pancreatic secretory responses to secretin and pilocarpine respectively, as shown by figure 4, gives the following contrasts. The general blood pressure and organ volume changes following the administration of pilocarpine, irrespective of the initial cardiac slowing, are directly opposite those obtainable with secretin. The splanchnic organ volume changes after pilocarpine follow the blood pressure almost exactly, while secretin, on the other hand, produces a passive vasodilatation of the splanchnic region similar to the nitrites.

The secretin secretory curve, in rapidly attaining a maximum and gradually decreasing to normal by approximately the 20th minute after administration, differs widely from that obtained with pilocarpine. With the latter, a longer latent period was observed, as previously shown by Bayliss and Starling (1904), the maximal secretion peak was reached later and the augmented secretion continued for 45 to 75 minutes.

By the administration of atropine it was possible to stop the pancreatic secretion induced by pilocarpine at any stage within a period of 30 to 90 seconds. The further administration of pilocarpine, following atropinization, had no demonstrable effect on the blood pressure or the pancreatic flow even though a total dosage of pilocarpine equivalent to 500 times that of the atropine, previously injected, was administered.

SUMMARY. It has been shown that the intravenous administration of several vasodilatins including sodium nitrite, Merck's peptone, Witte's and histamine results in a greater or less external secretion of the pancreas as compared with secretin. This pancreatic stimulation is paralleled by a greater or less fall in blood pressure, depending on the character and dosage of the vasodilatin injected, and an increased splanchnic blood flow and organ volume. The secretagogue response to sodium nitrite is not influenced by ligation of the pylorus, cutting the cervical vagi, or the administration of either atropine or ergotamine in large doses. As a consequence, the mechanism resulting in the stimulated pancreatic flow seems closely associated with the improved blood supply of the pancreas as a result of the general splanchnic vasodilatation.

It may be pointed out that a similar increase in the splanchnic organ

volume as well as pancreatic blood flow was observed following the introduction of dilute HCl into the duodenum (Burton-Opitz, 1920; Barlow, 1926) and likewise resulted from the vein administration of vasodilatin-free secretin. It is possible, therefore, that the splanchnic vascular change observed is one of the important chemical responses resulting from the normal expulsion of acid chyme into the duodenum (Pawlow, 1912), and consequently may be the intermediary or the end point of one of the "causes of the external secretion of the pancreas" (Ivy, 1926). Aside from the fact that a variety of substances, including nitrites, proteoses, cleavage products such as histamine and various intestinal mucosal extracts, produce a comparable vascular response as well as pancreatic secretory responses, a number of questions are raised as to certain physiological responses under conditions of hypotension.

The vascular and pancreatic secretory responses following the administration of pilocarpine have been correlated with similar data from comparable dosages of a standard pancreatic secretin. The splanchnic vascular changes observed paralleled very closely the general blood pressure curve; presumably, the splanchnic vessels were not affected noticeably by pilocarpine since no vascular response of any kind, within limits, was obtainable after atropine.

Pilocarpine stimulated the external pancreatic flow irrespective of ligation of the pyloric valve, as observed by Camas and Gley (1913). Since the vascular and pancreatic secretory responses following the injection of pilocarpine may be entirely inhibited by atropine, it appears that the point of attack of pilocarpine, in producing a copious external secretion of the pancreas, is not on the nerves of the intestine or even through the production of secretin as maintained by Hustin (1920), since the pancreatic secretion produced by secretin or even by placing dilute HCl in the intestine is wholly unaffected by the administration of atropine either by vein or subcutaneous injection (Bayliss and Starling, 1902; Farrell and Ivy, 1926).

CONCLUSIONS

1. It has been shown that vasodilatins per se are both additive and synergistic with standard pancreatic secretin preparations.

2. Peptone, histamine and sodium nitrite increase the external pancreatic secretory rate presumably by improving nutritional conditions, i.e., blood supply to the splanchnic organs, since the secretory rate subsequent to the administration of sodium nitrite was unaffected by cutting the cervical vagi, ligation of the gastric pylorus, or the administration of atropine or ergotamine.

3. The general and splanchnic vascular changes have been correlated with the pancreatic secretory rate following the administration of pilo-

carpine. Further proof of the mechanism of the pilocarpine secretagogue action has been presented.

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THE ADJUSTMENT OF HEART RATE AND ARTERIAL PRESSURE IN HEALTHY YOUNG WOMEN DURING PROLONGED STANDING

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The fall in circulatory minute volume noted in healthy young women on changing from a reclining to a quiet sitting or standing position (Turner, 1927) led to a further consideration of ways of testing the circulation. It appeared in the work referred to that while circulatory minute volumes show a wide range of response to changed positions among healthy young women the extent of the decrease noted in the quiet erect position is roughly indicative of the difficulty and success of maintaining that position. Those subjects whose minute volumes fell off most markedly on maintaining this quiet standing position were often the ones who gave other evidence of lessened circulatory efficiency by fainting, dizziness or a sense of extreme fatigue.

It therefore appeared desirable to estimate by some other and more simple procedure the efficiency of this circulatory adaptation. The results have shown that the holding of a *quiet* standing position through a period of fifteen minutes is a more difficult task than was thought at first. Persons are frequently found whose circulation adapts itself with apparent ease to strenuous exercise yet who find quiet standing more difficult, as is shown by their reactions both subjective and objective. However, since the subjects in the best physical condition, young women near the end of their training in a school of physical education who stood very easily and with no fatigue, showed a relatively slight fall or a rise of minute volume on sitting and a comparatively slight fall on standing, it seems probable that the best circulatory adjustment is one in which the minute volume of the reclining position is maintained during standing.

It has been known from the work of many observers and particularly from the determinations of Crampton's index (Crampton, 1913, 1915) and the Schneider rating (Schneider, 1920, 1923; Schneider and Truesdell, 1922, 1923) that immediately on standing the heart rate almost invariably rises while the systolic pressure may rise, remain unchanged or fall. Observations are lacking on the changes in heart rate and in systolic and diastolic

pressures which take place during a period of ten to fifteen minutes of quiet standing, a time corresponding to that used for the tests of circulatory minute volume previously mentioned. Barach and Marks (1913) made observations for five minutes of standing only, a time not long enough to be an adequate test of endurance in this position. It was therefore planned to make such observations systematically on the subjects used for the previous experiments and also to make the Schneider test since that seems by far the best of the tests previously devised to try out the success of circulatory adjustment. After this first set of observations on twenty four subjects had been made, a second series on forty additional subjects was completed.

METHOD. The subjects chosen were those on whom the circulatory minute volumes in reclining and standing had been determined by the Field-Bock method (Field, Bock, Gildea and Lathrop, 1924; Turner, 1927). As has been previously reported, they were young women in good health. One of the group became ill and was therefore omitted so this series numbers twenty-four.

The subject came to the laboratory three or more hours after the last meal, preferably in basal metabolic condition or late in the forenoon after an ordinary breakfast. The late afternoon rise in pulse rate and also the fatigue of the day were thus avoided. A Schneider test was given first, with the additional observation of diastolic pressure though this was not used in the Schneider scoring.

The test of the reaction to prolonged stan ding began by a control period of reclining, long enough for heart rate and arterial pressures to become reasonably steady. Observations of rate and pressures were made at three minute intervals and the duration of reclining made dependent on the finding. Twelve to fifteen minutes were usually sufficient for the securing of two successive sets of readings practically alike, and hence presumably indicative of the reclining circulation. The subject then rose quietly with as little exertion as possible and stood still for fifteen minutes during which time rates and pressures were observed at intervals of two or three minutes or sometimes even more frequently if significant changes were taking place. The subject then reclined for a final period of about ten minutes to check the findings of the preliminary reclining period. A stop watch was started when the subject reclined after the Schneider test and all records were kept with reference to the minutes indicated by this.

The same care was taken as in the experiments on circulatory minute volume to have the subject at ease and comfortable when reclining. No directions were given as to the mode of standing save the request to stand *still*. It was thought that this would give the standing result most natural for each subject. Effort was made to rule out disturbing factors which might alter the easily changed circulatory reactions. All rates were

counted for thirty seconds and recorded on the charts in beats per minute. In taking pressures a B-D mercury manometer was used and care was taken to get at each determination the upper limit of systolic pressure, which demands watchfulness in subjects who show a respiratory rhythm. This rhythm frequently amounted to 6 to 8 mm. of mercury. The mercury was allowed to fall slowly through the critical region, with the time of a complete respiration for about 2 mm. of fall. The diastolic pressure was taken at the beginning of the fourth phase, the point which seems sharpest for comparative determinations. The diastolic level showed less respiratory rhythm than the systolic. Since the test implies the taking of many successive determinations, the bag was held inflated for as short a time as was consistent with accurate results. In a few subjects the diastolic end-point was ill-defined. In a few others the pulse was hard to count, a fact associated apparently either with a low pulse pressure or with the conformation of the wrist. A stethoscope over the heart is a possible way out in such cases though not used in these experiments.

Discussion of results and scoring. The tests were made once or twice on each subject. All results were charted and figures 1 to 7 show the character of the records. The last determinations of the reclining period are taken as indicative of the reclining circulation. The work of rising introduces an additional factor into the record, hence the determinations taken immediately after rising are the result of both the change in position and the work of rising. The small amount of work involved in rising must however have only a transitory effect. It is thus to be expected that the first two or three minutes after rising may show fluctuations which tend to smooth out as the adjustment to the new position becomes established. A time three minutes after rising has been taken arbitrarily as indicative of the response to change in position. As standing is prolonged, further circulatory changes appear and as a test of the subject's endurance these changes have been studied for the period five to fifteen minutes after rising.

Certain details about this series of 33 tests are listed in tables 1 and 2. These tables group the subjects as "good" and "poor," according to the success of their previous minute volume experiments. Fifteen subjects in whom the fall in minute volume on standing was less than 28 per cent are called "good;" nine subjects where it was 28 per cent or more are called "poor." This is a purely arbitrary division based on the fact that subject K whose fall in minute volume on standing was 28.3 per cent grew dizzy while subject S whose fall was 27.9 per cent did not. All other subjects who showed outward evidence of circulatory embarrassment fall in the group below K.

The points noted in tables 1 and 2 are as follows:

1. The average heart rate for the reclining period in all the long tests on circulatory minute volumes. For good subjects this averages 60.8 beats per minute, for the poor subjects 69.8.

2. The percentage change in minute volume on standing, taken from the long experiments. For good subjects this averages −9.5 per cent, for the poor subjects −40.1 per cent.

3. The Schneider ratings. For good subjects 12.9, for poor subjects 9.0.

4. The reclining heart rate taken from the last determinations of the preliminary reclining period in the tests of this series. For the good subjects this averages 63.3, for the poor subjects 70.8. These are higher than in 1, because all subjects were not in basal metabolic condition and some experiments were later in the day.

5. The rise in heart rate on first standing, taken by comparing 4 with the record three minutes after rising. For the good subjects this averages 18.0 beats per minute, for the poor subjects 19.2.

TABLE 1

Data on heart rates in "good" subjects in standing experiments on circulatory minute volumes and on the same subjects during prolonged standing

SUBJECT	C. M. V. EXPERIMENT		NUMBER EXPERIMENT	SCHNEIDER RATING	PROLONGED STANDING TEST		REMARKS
	Average H. R. reclining	Change in C.M.V. on standing			H. R. reclining Last determination of preliminary period	Change in H.R. 3 minutes after rising: beats per minute	
A	59.2	−6.2	75	8	78	+19	Late p.m. Warm
B	60.5	+28.5	89	13	58	+32	Two C. M. V. experiments
B	60.5	−14.6	118	12	58	+30	Warm
D	55.7	−12.4	67	11	76	−1	Late p.m. Low thyroid?
E	58.3	−5.4	70	15	60	+12	
G	56.8	−1.7	79	10	74	+11	Late p.m.
I	68.3	−14.3	78	12	64	+24	
J	59.4	−5.8	66	14	58	+16	
M	64.3	−9.0	76	15	56	+19	
M	64.3	−9.0	117	15	60	+31	
O	65.8	−9.5	74	14	66	+2	Late p.m.
O	65.8	−9.5	119	13	62	+14	
P	60.0	−5.1	73	17	54	+20	
Q	61.7	−16.3	65	16	60	+24	
R	61.9	−1.4	71	10	68	+27	
S	62.0	−27.9	69	13	64	+18	
X	55.7	−25.9	132	14	50	+17	
Y	63.2	−24.6	113	10	74	+9	
Average	*60.8*	*−9.5*		*12.9*	*63.3*	*+18.0*	

"Good" subjects: Fall of less than 28 per cent in circulatory minute volume in standing.

H.R. Heart rate.

C.M.V. Circulatory minute volume.

In addition to this evidence regarding rates it was noted that there were in the records for the fifteen good subjects only five cases of abrupt change in rate (ten or more beats per minute) after the first three minutes of standing while in the smaller group of nine poor subjects there were seven such instances.

TABLE 2

Data on heart rates in "poor" subjects in standing experiments on circulatory minute volumes and in the same subjects during prolonged standing

SUBJECT	C. M. V. EXPERIMENT		NUMBER EXPERIMENT	SCHNEIDER RATING	PROLONGED STANDING TEST		REMARKS
	Average H. R. reclining	Change in C.M.V. on standing			H. R. reclining. Last determination of preliminary period	Change in H. R. 3 minutes after rising; beats per minute	
C	77.9	−49.2 −41.2	68	10	80	+15	Two C.M.V. experiments
F	71.5	−30.6	77	2	80	+13	Late p.m.
F	71.5	−30.6	120	8	74	+17	
H	65.0	−54.9	84	14	66	+8	Low thyroid? After thyroid tablets
H	65.0	−54.9	114	11	68	+16	
K	76.7	−28.3	72	6	78	+16	Late p.m.
K	76.7	−28.3	115	7	78	+24	Dizzy
L	63.3	−34.7	80a	6	66	+33	Faint
L	63.3	−34.7	80b	6	68	+4	Immediately after 80a
L	63.3	−34.7	166	13	52	+31	Favorable circumstances
T	66.5	−32.8	85	9	74	+18	Forenoon
T	66.5	−32.8	86	8	78	+15	Late p.m.
U	79.0	−20.5*	99	7	76	+32	Incomplete. Fainted
V	64.5	−50.7	103	16	60	+16	Inconsistent with C.M.V. experiment
V	64.5	−50.7	111	13	68	+18	
W	63.7	−38.7	116	8	66	+27	
Average	*69.8*	*−40.1*		*9.0*	*70.8*	*+19.3*	

"Poor" subjects: Fall of 28 per cent or more in circulatory minute volume on standing.

H.R. Heart rate.

C.M.V. Circulatory minute volume.

* Experiment incomplete. End result undoubtedly lower.

With regard to *rate*, then, it appears that the subjects who had shown better reactions in their volume experiments showed here conspicuously lower rates reclining, slightly less rise on standing and markedly greater steadiness as standing continued. The best type of reaction seemed to be a reclining rate of not more than 60, a rise on standing of 10 beats or less, with little or no subsequent change as standing continued during fifteen

minutes. In all cases the second period of reclining showed a speedy return to approximately the previous reclining value. Such an evaluation of the rates is in accord with the work of many previous observers.

The *pressure* determinations lend themselves less easily to tabulation. There is practically no difference between good and poor groups in reclining systolic, diastolic and pulse pressures. The subjects were healthy young women and there are thus no cases of notably high or low pressures. A few showed a reclining systolic pressure between 96 and 99 mm., three in the good group and five in the poor group. The reclining pulse pressure varied much, from 34 to 52 in the good group, with an average of 43.1, and from 22 to 50 in the poor group with an average of 37.9. The tendency toward a lower pulse pressure in the latter group may be significant. The average for *systolic* pressure in the good group remained unchanged on standing and fell slightly in the poor group. A conspicuous fall was considered a poor indication, as in the Schneider rating. As standing continued the systolic pressure was maintained in the good subjects but dropped in those who were poorest. In some instances an initial rise, perhaps induced by the work of rising, gave way to a fall later on. The *diastolic* rise three minutes after standing is almost the same in the averages for the two groups. Later on the diastolic pressure shows only minor changes in good subjects while in the poorest the rise continues until there is little pulse pressure left or a fall appears, coincident with a fall in systolic pressure. In these last cases dizziness and fainting occurred. The *pulse pressure* decreased in every case on standing. With only two exceptions this was due more to the rise of the diastolic than to the fall of the systolic pressure. The average decrease for the good subjects was 13.1 mm. and and for the poor subjects 14.7 mm. In one instance the diastolic rise did not occur, perhaps by reason of peripheral dilatation due to the extreme heat and humidity of the day. In this instance the systolic pressure fell markedly and the subject became almost, perhaps quite unconscious. If large decreases in pulse pressure occur, the reaction appears unfavorable. These results are in general accord with those of Barach and Marks (1913), though their period of standing was but five minutes. There is apparent in some cases an effort to save the unfavorable situation associated with a greatly lessened pulse pressure by a very rapid heart rate. If this expedient fails, the subject is in straits, but as long as the rate continues high some subjects, for instance *T* (see fig. 3), seem able to keep on standing without subjective symptoms in spite of a very low pulse pressure. The few subjects in which this was true were all accustomed to standing, but whether the two circumstances are related in any way is not clear.

In order to get some approximate estimate of the indications given by this test, the findings were graded somewhat as in the Schneider test. The same total score, 18, was used, distributed among six factors, each of which

might receive a grade of from +3 to −3. Rate as a whole received a possible score of 9, pressure the same, with the various values planned as in table 3.

TABLE 3

Scoring for prolonged- standing test of circulatory adjustment

I. Heart rate	Possible score +9 to −9
a. Initial rate, reclining, after a period long enough for rate to become steady	Possible score +3 to −3
60 or below	+3
61 to 69	+2
70 to 74	+1
75 to 79	0
80 to 84	−1
85 to 89	−2
90 or more	−3
b. Rise on standing, read from plotted curve three minutes after rising	Possible score +3 to −3
10 or less	+3
11 to 20	+2
21 to 30	+1
31 to 40	0
41 to 50	−1
51 to 60	−2
61 or more	−3
c. General course of rate during prolonged quiet standing. Read from plotted curve for minutes 5 to 15 of standing period	Possible score +3 to −3
First rise on standing maintained	
Very steady, variations of not more than 2 beats per minute	+3
Steady, fluctuations 3 to 6 beats	+2
Fluctuations or rising tendency of 7 to 10 beats per minute	+1
Large fluctuations or rising tendency or more than 10 beats per minute	0
Decreasing tendency	
Decreasing rate with pressure score of 5 or more	+2
Pressure score 4 or less	
Decrease not more than 6 beats from highest point	−1
Decrease 7 to 12 beats from highest point	−2
Decrease 13 beats or more from highest point	−3
If subject has to cut standing period short and rate can not be gotten for complete time	−3
II. Arterial pressure	Possible score +9 to −9
a. Systolic pressure	Possible score +3 to −3
1. General level, reclining, usually no score for healthy subjects	
If above 130 or below 95 mm	−1
(Not used if systolic score is −3)	
2. Change on standing, read from plotted curve 3 minutes after rising	
Rise or no change	+1
Fall of 1 to 4 mm	0
Fall of 5 mm. or more	−1

TABLE 3—*Concluded*

3. General course during prolonged quiet standing.
   Read from plotted curve for minutes 5 to 15 of standing period
   Very steady, variations of not more than 2 mm..... +2
   Steady or general rising tendency, fluctuations of 3 to 6 mm.... +1
   Fluctuations of more than 6 mm. or falling tendency to not more than 8 mm. below highest level..... 0
   Falling by 9 to 12 mm. from highest standing level..... −1
   Falling by 13 mm. or more from highest standing level..... −2
   If subject has to cut standing period short and determinations can not be gotten for complete time..... −2

b. Diastolic pressure.....Possible score +3 to −3
1. Change on standing, read from plotted curve 3 minutes after rising
   No change or rise
   If the pulse pressure does not become less than 20 mm..... +1
   If pulse pressure becomes less than 20 mm..... 0
   Rise of 6 mm. or less if systolic falls..... 0
   Fall..... −1
2. General course during prolonged quiet standing.
   Read as systolic pressure
   Very steady, no variations of more than 2 mm..... +2
   Steady, fluctuations of 3 to 6 mm..... +1
   Fluctuations of more than 6 mm. or rising tendency to not more than 8 mm. above lowest standing level
   If pulse pressure 20 mm. or above..... 0
   If pulse pressure below 20 mm..... −1
   Rising by 9 mm. or more..... −1
   Falling by 9 to 12 mm..... −1
   Falling by 13 mm. or more..... −2
   If the subject has to cut standing period short and determinations can not be gotten for complete time..... −2

c. Pulse pressure.....Possible score +3 to −3
1. Minimum score
   Never below 20 mm. during entire 15 minutes of standing..... +1
   One record below 20 mm. and that corrected in part or wholly... 0
   Remaining below 20 mm. for more than one determination..... −1
2. General course during prolonged quiet standing.
   Read as systolic pressure
   Very steady, no variations of more than 4 mm.....+2
   Steady, fluctuations of 5 to 8 mm..... +1
   Fluctuations through more than 8 mm..... 0
   Falling by not more than 12 mm. from highest standing level
   If minimum 20 mm. or above..... 0
   If minimum below 20 mm..... −1
   Falling by 13 to 20 mm. from highest standing level
   If minimum 20 mm. or above..... −1
   If minimum below 20 mm..... −2
   Falling by more than 20 mm..... −2
   If subject has to cut standing period short and determinations can not be gotten for complete time..... −2

Notes to Table 3.

Note 1. Plotting the determinations is essential for getting a picture of the progress of the various changes.

Note 2. The scoring has been made for young women in good health. The test has not been used on men, on women beyond 37 years of age, or on pathological subjects. The scoring for such groups of subjects would need modification. Such modifications would be, however, easy to plan after a few preliminary tests on the groups in question.

Note 3. A fluctuation is defined as a change which is followed by one in the opposite direction and which does not seem to affect the general level of rate or pressure. It may be due to influences beyond the control of the observer, such as mental states or slight involuntary or voluntary movements.

Table 4 gives the results of these scores, with the subjects grouped as before, into "good" and "poor." The results of the Schneider test, taken just before the prolonged standing test, are included. The column headed "Remarks" adds some pertinent data with respect to either subjects or experiments. Like the Schneider tests these are affected by the diurnal circulatory rhythm though not to the same degree. The subjects whose low heart rate may possibly be associated with a hypothyroid condition are indicated, also those in whom circulatory embarrassment was evident and those whose tests failed to be consistent with the longer experiments on circulatory minute volumes.

In the main it is clear that the good subjects, by the former criterion of a moderate fall in circulatory minute volume on prolonged standing, are likewise the ones who show a good or a fair score by this test, while the poor subjects receive much lower scores on rate and pressure. The averages for the good and poor groups are, for the Schneider test, 12.9 and 9.0; and for this test, 9.7 and 4.1. It appears that prolonged standing tends to separate the two groups farther than the combination of brief standing and exercise used by Schneider, and for two reasons: 1, progressive changes take place in the poorer subjects as quiet standing is prolonged, and 2, evidence furnished by diastolic and pulse pressures is given equal importance with that from systolic pressure.

Figures 1 to 7 illustrate the curves plotted from the data of these pulse rate and arterial pressure tests and serve to make the nature of the evidence clearer. The legend for each figure comments upon the experiment. Table 5 gives details of the scoring.

To test the matter further, the heart rates and pressures were determined by the same method upon a new series of forty subjects. None of these women had been subjects for the minute volume experiments and many of them were unfamiliar with this type of study. They were very like the first group in age, normal build, presumable good health and so on, though this group includes no women highly trained physically. There is among them a large group of nurses who stand for rather long periods in the course of their experience, particularly in the operating room. Table 6 gives the

TABLE 4

Summary of results of scoring subjects of circulatory-minute-volume experiments on heart-rates and arterial pressures during prolonged standing

	SCHNEIDER RATING	STANDING TEST	REMARKS
Good subjects			
A	8	+5	Late p.m.
B	13	+2	Difficult subject to interpret
B	12	+11	Inconsistent with C.M.V. expt.
D	11	+13	Late p.m. Low thyroid?
E	15	+14	
G	10	+9	Late p.m.
I	12	+5	Inconsistent with C.M.V. expt.
J	14	+13	
M	15	+12	
M	15	+3	
O	14	+16	Late p.m.
O	13	+9	
P	17	+14	
Q	16	+11	
R	10	+5	Inconsistent with volume experiment
S	13	+12	Late p.m.
X	14	+9	
Y	10	+11	
Average for 18 experiments	*12.9*	*+9.7*	
Poor subjects			
C	10	0	
F	2	+1	Late p.m.
F	8	+8	Better conditions than preceding experiment
H	14	+11	Low thyroid? Results better than in volume experiments
H	11	+12	
K	6	+6	Late p.m.
K	7	−4	Dizzy, standing period cut short
L	6	−10	Faint, standing period cut short
L	6	+6	Second trial, after faint. Short experiment
L	13	+3	Better conditions than preceding experiment
T	9	+4	Late p.m.
T	8	+6	Fainted, standing period cut short
U	7	−6	Better than volume experiments
V	16	+16	
V	13	+4	
W	8	+8	Difficult subject to interpret
Average for 16 experiments	*9.0*	*+4.1*	

rating for this series of tests, both for the Schneider test and that of prolonged standing, with remarks about the experiments. The table shows two groups: 1, those subjects regarding whom there was no adverse evidence in personal history or during the course of the experiment as dizziness, fainting, cyanosis, poor history for standing, exercise restricted because of cardiac limitation (though no marked defect) and so on, and 2, those for whom some such evidence appeared. All these tests were made in hot weather, July. Undoubtedly the heat was a factor in at least some of the results. Tests made when the room was distinctly uncomfortable from heat or humidity or both are indicated by "hot day" under remarks. In several instances experiments were repeated under more favorable conditions, and with the provision that the subject rose slowly if she were habitually dizzy on first standing. It will be seen in the table that the

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Fig. 1. Subject O, experiment 74, score +16.

This record shows remarkable steadiness in both pulse and pressure after the adjustment on first assuming the standing position. The rate rises very little and remains steady. The systolic pressure falls slightly but it remains steady without further fall as standing is continued. The usual rise in diastolic pressure fails to occur but from the maintenance of the systolic pressure and the steady low rate it is clear that the circulation is well-adjusted.

Subject O was a student at the Boston School of Physical Education and stood in beautiful position with no restlessness or fatigue.

Age, 21; height 164.5 cm.; weight 65.2 kgm.

Fig. 2. Subject Y, experiment 113, score +11.

This subject shows a rather slow adjustment to the standing position but the adjustment once made is maintained to the end of the standing period with only a slight falling off which may be merely a fluctuation. The initial pulse rate is higher than in subject O, however, the rise in rate on standing is greater and the systolic and pulse pressures fall at that time. The diastolic rise is about as seen in many good subjects.

Subject Y was a secretary of fair posture, squarely built.

Age, 36; height, 156.1 cm.; weight, 60.5 kgm.

Fig. 3. Subject T, experiment 85, score + 4.

The record shows a distinctly high rate on standing and this continues to rise. The diastolic pressure shows a marked rise and the systolic an evident though a slight fall. The pulse pressure is very low throughout the period of standing. This subject is accustomed to standing in laboratory; she said she was not dizzy in this experiment but her history includes unexplained dizzy attacks. The chart indicates that if either the heart beat or the vascular constriction fell off she would undoubtedly suffer. As it is, the heart's output must be greatly reduced, so much so that one wonders how long the cerebral and coronary circuits would have been adequately supplied. Her record for circulatory minute volumes shows a fall in the last period of the standing test of 47 per cent from the reclining value, quite in harmony with the great fall indicated by the present record. The rise of pulse in the long experiment was from 66.7, the reclining average, to 95 in the last standing determination, again like the reaction in the present experiment.

Subject T was a graduate student in physiology, tall and slender, with hollow back.

Age, 28; height, 170.5 cm.; weight, 59.2 kgm.

Fig. 4. Subject K, experiment 115, score −4.

The record shows a high preliminary heart beat which rises markedly on standing. Contrary to the history of subject T, the rate here fell at minute 26 and further at minute 27.5, 11 and 12.5 minutes after rising respectively. The systolic pressure, too, was not maintained. The subject was so dizzy that she was obliged to lie down at minute 29.5, when the circulation at once readjusted itself. An earlier test on this subject showed very great fluctuations in both rate and pressure with an apparent inability to maintain a steady state when standing.

Subject K was a college student of fair posture. Not at all athletic.

Age, 17; height, 163.4 cm.; weight, 57.9 kgm.

Fig. 5. Subject C, experiment 68, rating 0.

The entire experimental record of this subject is poor. In this test during the later part of her standing period it was almost impossible to get either pulse at the wrist or sounds in the stethoscope, particularly the diastolic level. While the subject got through this experiment without dizziness, she is subject to it on standing. She stood on this occasion far from still, though the movements were unconscious and there were frequent deep sighs. The record shows a high preliminary pulse, a continued rise during standing with a fall at the last determination probable. The systolic pressure falls and the diastolic rises excessively so that the pulse pressure is greatly reduced.

Subject C was a college student, tall and slender, of very poor posture, with hollow back and protuberant abdomen.

Age 25; height, 167.4 cm.; weight, 62.0 kgm.

Fig. 6. Subject U, experiment 99, rating −6.

This subject is one in whom the early adverse indications were less marked than in subject 6. The experiment terminated abruptly as the pressure was being taken at minute 20.5 because the subject fainted. Previous unfavorable indications had been a very rapid heart rate and a falling systolic pressure. The heart rate probably failed suddenly for the count at two minutes after she was put on the couch was very low for her customary reclining rate. She did not regain consciousness until the head was lowered well below the body. The necessary readjustment of apparatus delayed the taking of pressures until after the interesting period of recovery was over.

Subject U was a college instructor in biology, of good posture but not of rugged health. Never in athletics. Dark circles under eyes frequent. The day of this experiment was hot.

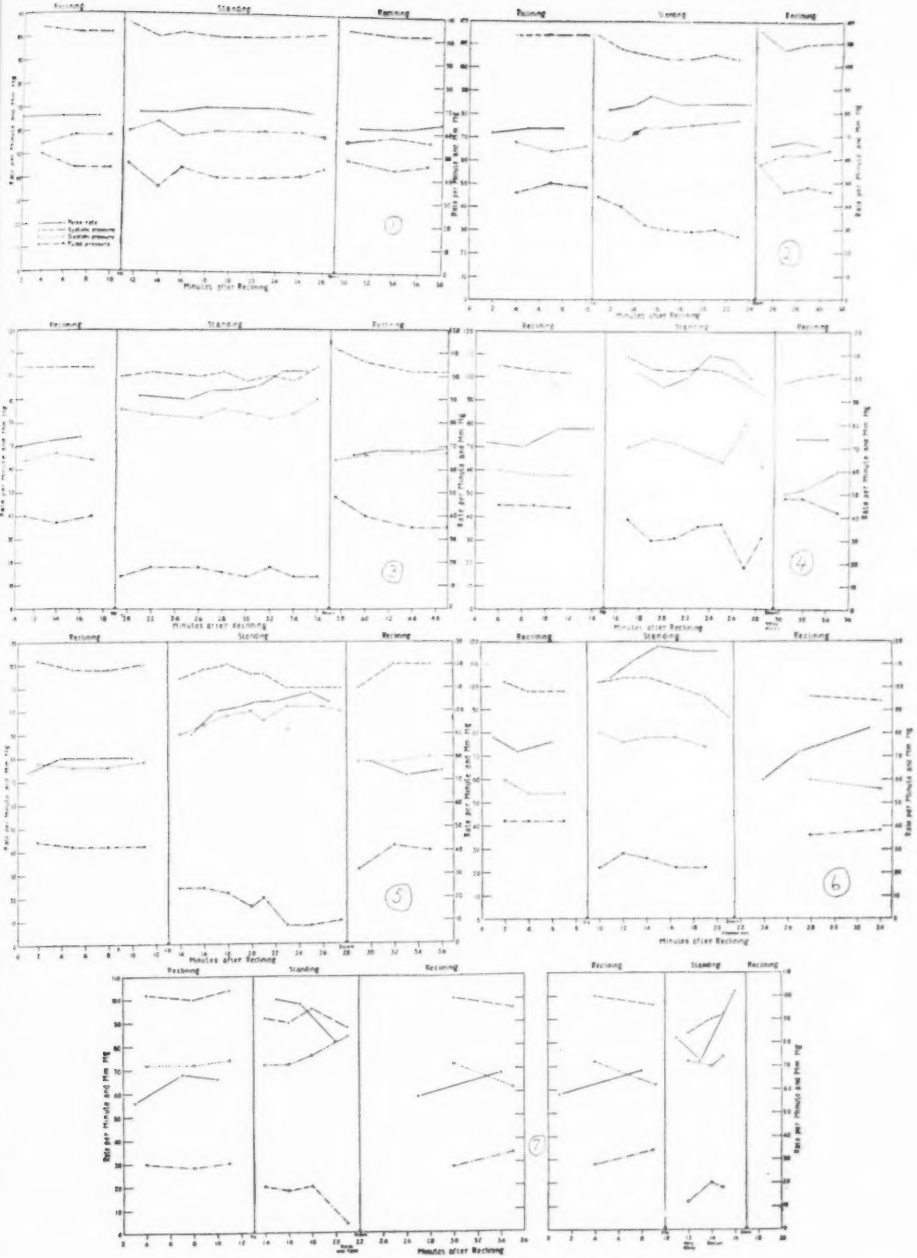
Age, 24; height, 165.1 cm.; weight, 50.1 kgm.

Fig. 7. Subject L, experiments 80a and 80b, scores −10 and + 6.

Experiment 80a was abruptly terminated by the dizziness and faintness of the subject. The pulse rate dropped rapidly, the systolic pressure fell, the diastolic rose after a delay and the pulse pressure all but disappeared. The subject was willing to try the experiment again. On the second attempt she showed again a falling rate after the first rise and a low systolic pressure, with great dizziness, but her condition improved in that both rate and systolic pressure rose and the dizziness disappeared. How long this would have lasted is not known since there was no opportunity to finish the experiment because of an imperative engagement. The day was very hot and humid. It would appear that the usual diastolic rise was impossible in experiment 80a because of skin dilatation due to the heat. Another record taken on a cooler day and when the subject was less fatigued gave a score of +3. There was the ordinary diastolic rise but the systolic pressure fell and the rate rose more than in good subjects.

Subject L was a graduate student and laboratory assistant in physiology, of poor posture.

Age, 22; height, 166.8 cm.; weight, 57.8 kgm.


Reclining
Standing
Reclining
Pulse rate
Systolic pressure
Diastolic pressure
Pulse pressure
Rate per Minute and Mm Hg
Minutes after Reclining
1
2
3
4
5
6
7

averages for the good and poor groups are: Schneider test, 9.1 and 6.9; this test, 8.3 and 1.6. As in the first group of subjects the ratings for this test separate more widely than for the Schneider test.

A detailed study of records, individually and by tabulations, is necessary in order to show approximately the volume changes and the success with which the circulatory apparatus is meeting the call upon it for a difficult adjustment. It is clear that nearly all subjects respond to the brief standing and to the small amount of exercise called for by the Schneider test

TABLE 5

Details of scoring of tests charted in figures 1 to 7

	SUBJECT, NUMBER OF EXPERIMENT							
	O-74	Y-113	T-85	K-115	C-68	U-99	L-80a	L-80b
Rate								
Initial	+2	+1	+1	0	−1	0	+2	+2
First rise	+3	+3	+2	+1	+2	0	0	+3
General course	+3	+2	0	−3	+1	−3	−3	0
Pressure								
Systolic								
First change	0	−1	0	+1	0	+1	−1	−1
General course	+2	+2	+1	−1	−1	−2	−2	+1
Diastolic								
First change	+1	+1	0	+1	+1	+1	−1	0
General course	+2	+1	−1	−1	+1	−2	−2	+1
Pulse pressure								
Minimum	+1	+1	−1	0	−1	+1	−1	−1
General course	+2	+1	+2	−2	−2	−2	−2	+1
Total score	+16	+11	+4	−4*	0	−6†	−10‡	+6(?)§

* Subject obliged to recline after 14.5 minutes standing. Dizzy. Last standing pulse count not obtained.

† Standing period 13 minutes. Subject fainted. Both pulse and pressure records thus incomplete.

‡ Standing period 9 minutes. Subject dizzy and faint. Both pulse and pressure records thus incomplete.

§ Standing period only 7 minutes. Subject not dizzy. Record incomplete but not due to poor condition of subject. See figure 7.

more uniformly and more successfully than to the prolonged standing. It is in the later part of the test that the differentiation between subjects becomes more apparent, as those in whom the adjustment is less successful show progressively worse indications in rate and pressure, culminating in the least good cases in subjective dizziness or in fainting.

It may be suggestive to think of the essentially difficult task of the circulation as that of getting blood *to* and not *from* the heart. In the quiet erect position the usual aids to the return of blood to the right heart during

exercise are lacking, there is no rhythmic muscular motion, there is little increase of thoracic aspiration. On the other hand there is because of gravity a difficulty in the return of the large amount of blood below the

TABLE 6

Summary of results of scoring 40 new subjects on heart-rates and arterial pressures during prolonged standing

	SCHNEIDER RATING	STANDING TEST	REMARKS
		Good subjects	
Bem	12	+7	Hot day
Chr	2	+10	Hot day. Subject used to standing
Chr	−2	−1	
Col	17	+15	
Cor	16	+8	
Dod	17	+13	
Dar	9	+5	Late p.m. Brief poor reaction Used to standing
Eas	7	+5	
Eme	9	+11	
Eve	11	+8	Late p.m. Hot day
Fla	9	+3	Late p.m. Hot day
Har	12	+9	
Hat	13	+13	Late p.m.
Hil	14	+9	
Hil	15	+4	
Hol	8	+11	Late p.m.
Hol	14	+7	
Hop	5	0	Used to standing
How	11	+9	
How	13	+10	
Mas	3	+5	
McN	9	+8	Hot day. Late p.m.
One	13	+11	
Pho	13	+10	Hot day
Ros	10	+9	Hot day
Saw	6	+6	
Squ	7	+10	Hot day. Late p.m.
Ste	10	+14	
Tur	11	+10	
Wal	16	+17	
Wal	16	+14	
War	13	+11	Late p.m. Hot day
Was	6	+8	Late p.m.
Woo	10	+3	Late p.m. Hot day
Average for 34 experiments	*9.1*	*+8.3*	

TABLE 6—*Concluded*

	SCHNEIDER RATING	STANDING TEST	REMARKS
		Poor subjects	
Car	9	−1	Hot day. Nearly fainted. Standing period cut short
Fin	7	−2	Cyanosis of face conspicuous
Hay	9	+5	Hot day. Very dizzy. Standing period cut short
Hay	12	−4	Cooler day. Rose slowly. Not dizzy
Ken	5	+3	Exercise restricted from heart Hot day. Not dizzy in this experiment
Lew	13	+8	Dizzy. Very hot day. Late p.m. Standing period cut short
San	7	+5	Felt "short of air." Late p.m. Hot day
Stu	3	−8	Hot day. Dizzy and faint. Standing period cut short
Stu	11	+8	Cool day. Stood slowly. Not dizzy
Wes	3	+6	Not dizzy. Hot day. Restricted exercise. "Rheumatic heart"
Wil	3	−9	Dizzy. Hot day. Late p.m. Standing period cut short
Wil	8	+1	Cooler day, a.m. Not dizzy
Knu	4	+3	Faints at dressmaker's. Not dizzy in this experiment
Wel	2	+7	Exercise restricted from heart Not dizzy in this experiment
Average for 14 experiments...	*6.9*	*+1.6*	

level of the heart. It is possible that the vertical abdomen with its unsupported ventral wall may account for the unsuccessful return of blood to the heart at least in some persons. It is conspicuous that the young women who stood most easily and successfully from the standpoints of objective appearance and lack of fatigue were those in whom there was a slow rate and a relatively large pulse pressure, indicating a heart adequately filled between beats. In the longer experiments on circulatory minute volume a great fall in the heart's output on prolonged standing was associated with a rapid beat. It seems that the inadequately filled heart attempts to maintain an adequate pressure by an increased rate. Thus the low pulse pressure and the rapid rate become indicative of a low output per minute.

That the return of blood to the heart is a most essential feature in circulatory success is indicated also by the trend of the work on vital capacity, especially that on residual air and on "functional residual air" by Binger and Brow (1924). The experiments on the young women above described indicate a failure in return to the right heart, while the work of Peabody (1917), Peabody and Wentworth (1917) and Peabody and Sturgis (1921, 1922) emphasizes for cardiac subjects the effect of a hold back in the lungs and hence a failure in the supply of the left heart.

To establish the effect of a little rhythmic motion a few subjects, after they had stood for a considerable time, were given a minute's exercise of stepping in place. Rate and pressure were determined as soon as possible afterward. Both rate and systolic pressure rose and diastolic pressure fell, a combination indicating an increased output of blood and hence its increased return to the heart.

These experiments thus indicate the general validity of the relation between the heart's output and the product PP $\times$ PR (pulse pressure $\times$ pulse rate) (Erlanger and Hooker, 1904, discussed by many others).

In conclusion, this study emphasizes the value of the standing position as a means of discriminating between the thoroughly adjustable circulation and one which may be indeed adequate for the demands of a considerable amount of exercise but which fails in its power to meet the demands of prolonged standing which are more difficult of accomplishment. Adequate circulatory response to the demands of exercise has been a matter of life and death through the ages of our ancestral life. The maintenance of a quiet erect position would not have been a problem before the erect position was assumed as habitual and indeed quiet in that position can scarcely ever have been of life-saving significance. Thus the reaction we have chosen for study is one probably recent in race history and less stabilised by necessity than the circulatory response to exercise. It is not unnatural to find here a differentiation between circulatory adjustments in different persons all reasonably healthy. An entirely successful meeting of the circulatory demands of prolonged standing may be an achievement which is reached only by an apparatus capable of the most perfect and responsive adjustment to relatively slight stimuli.

SUMMARY

1. A system of scoring, based on observations of heart rates and arterial pressures during a prolonged period of quiet standing and control periods of reclining, has been devised which appears to give an index of the ability of the circulation to adapt itself to changes in position.

2. The results of tests upon twenty-four healthy young women indicate a fair degree of parallelism between the scores by this test and measurement of the circulatory minute volume by the Field-Bock method. The

Schneider ratings were also frequently in agreement, though the new scores spread out more widely and thus serve to differentiate the subjects more clearly. There were some cases of discrepancy.

3. The heart rate and arterial pressure determinations were tried on a series of forty additional subjects with results which tended to corroborate those of the first series.

4. The use of the change from reclining to a more or less prolonged period of standing still is suggested for circulatory studies as likely to produce differential results quite as suggestive as those of exercise versus rest. Reasons for this may lie in the essential mechanical difficulties involved in maintaining the erect position and in its probable phylogenetic history.

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NOTE. After this paper went to press, a paper by Helmreich, 1923, was found. Eight well children, aged 8 to 15, were used as subjects in experiments to determine the influence of body position and muscular activity upon heart rate and oxygen consumption. It was found that when sitting, standing and kneeling were quietly maintained the excess heart rate as compared with reclining was much greater than in graded exercises where the oxygen consumption showed a much higher rise. The author therefore distinguishes two kinds of heart acceleration, static and dynamic, the former related to changed position and to an apparent decrease in circulatory minute volume, the latter related to the increased circulatory minute volume ordinarily associated with exercise. The use of a tilting board showed, further, that the pulse continued low when the head was below the level of the body and the return of blood to the heart from legs and abdomen was therefore favored. The metabolism was increased in this head down position especially when the angle was as much as 60°. No determinations are given for arterial pressures.

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THE EFFECT OF TURNIPS AND TURNIP JUICE ON THE BLOOD SUGAR, PHOSPHORUS AND CHOLESTEROL OF RABBITS

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A Chinese proverb says: "If you eat turnips followed by hot tea the doctors will be so disappointed that they will have to crawl over the street." In Central Europe and Russia the turnip (*Raphanus sativus*) is one of the common remedies used by the laity. In 1913 Grumme (9) and Engels (8) showed its excellent effect in cases of cholelithiasis. For these reasons it seemed worth while to study the effect of the Chinese turnip on the blood sugar, phosphorus and cholesterol in rabbits.

There are many varieties of turnip in China. They are named according to their color, size, time of planting or time of harvesting (17). But their dietetic and physiologic effects may be considered essentially the same (15). The juice of the red and green Chinese turnips was found by the writer to have the same pharmacologic action and to contain the same amount of copper-reducing substances. The color of green turnip juice changes to red on standing a short time.

PROCEDURE. Freshly washed Chinese turnips were rubbed on a grater, the juice pressed out through a cloth and filtered in the ice-box through filter paper until clear. From the turnip about 25 per cent of juice was obtained, containing an average of 0.65 per cent of nitrogenous substances (determined by the Kjeldahl method) and from 0.4 to 0.5 per cent of copper-reducing substances (determined by the Folin-Wu blood sugar method in protein-free filtrates).

For experiments in the line of blood sugar and phosphorus, normal rabbits of approximately 2.5 kgm. body weight were kept on a diet of boiled millet and raw cabbage. The rabbits were starved for 24 hours before each observation and starvation was continued during the experiment. As a rule, 20 cc. of freshly prepared raw turnip juice were injected subcutaneously. Turnip juice, boiled for one minute, was also used. Blood was taken from the marginal ear vein before and at various intervals after the injection. The blood sugar was determined by Folin and Wu's method and the inorganic phosphorus according to Benedict and Theis. When the blood was taken the rabbits were kept quiet in a tray. Moderate bleeding

performed with care does not affect the blood sugar, as shown by the writer in a previous paper (11).

The experiments on cholesterol were performed on rabbits under analogous conditions except that the rabbits were not starved either before or during the experiment. The effect of turnip on the blood cholesterol was studied on rabbits fed on millet and cabbage and injected subcutaneously with the turnip juice, and also on rabbits fed on raw turnip.[1]

Results. The subcutaneous injections of turnip juice did not give any sign of inflammation at the site of injections. The injections of raw turnip juice resulted in a rise of the blood sugar, in a drop of the inorganic phosphates and in a drop of the cholesterol, as can be seen from the tables. Boiled turnip juice, injected subcutaneously, was followed by a rise in the blood sugar and the inorganic phosphates. Raw turnip juice and adrenalin injected simultaneously (but separately, on the two sides of the chest) gave adrenalin-like sugar and phosphate curves.

Feeding raw turnip as a single food resulted in a marked drop of the blood cholesterol, as can be seen from table 3. In rabbit VIII the initial blood cholesterol was abnormally high (141 to 138 mgm. per cent). It persisted for three days of turnip feeding and went down only on the fourth day. In the following three days the blood cholesterol dropped to normal in spite of the change of the food to millet and cabbage. In rabbits IX and X the feeding of turnip resulted, in three and two days, in a marked drop of the blood cholesterol (to 32 and 46 mgm. per cent). But such abnormally low figures did not persist in spite of the continued feeding of turnip and one day later the blood cholesterol went up to 57 and 68 mgm. per 100 cc. In rabbit IX the cholesterol was further restored to normal in three days of millet and cabbage feeding.

Discussion. *Blood sugar.* The rise in the blood sugar of the rabbits following the subcutaneous injection of turnip juice might at first glance be attributed to its copper-reducing substances. But the average amount of these in 20 cc. of turnip juice is only 0.1 gram, which is evidently too small an amount to have any effect. The author confirmed this statement by direct experiment on a rabbit, the blood sugar of which showed only a slight rise (5 mgm.) in two hours following subcutaneous injection of 0.1 gram glucose in 20 cc. of water. Collazo (5), after injecting 5 grams of glucose in a dog, obtained only a slight rise, persisting at most for two hours. Potato juice containing the same amount of copper-reducing substances as the turnip juice had no such effect, as was found by the author in experiments on rabbits performed under analogous conditions.

The hyperglycemic action of turnip juice is evidently due to some

[1] The Chinese turnip contains, according to Adolph (1), 1.68 per cent of protein and 2.94 per cent of nitrogen-free extract.

substance which resembles in this respect the secretins of the vegetable kingdom which were described by Bickel (3) and his co-workers in spinach, Urtica dioica, asparagus, lettuce and strawberries, and are evidently wide-

TABLE 1

Effect of subcutaneous injections of turnip juice on blood sugar

TIME	PROCEDURE	SUGAR	DIET
		mgm. per cent	
April 1	Rabbit VII*		
9:25 a.m.	Control	102	
9:30 a.m.	10 cc. raw turnip juice		
10:00 a.m.		111	
11:00 a.m.		111	
12:00 noon		111	
2:00 p.m.		166	
April 6	Rabbit I*		
8:55 a.m.	Control	100	
9:00 a.m.	20 cc. raw turnip juice		
10:30 a.m.		200	
12:00 noon		200	
2:00 p.m.		200	
4:00 p.m.		200	
April 7			
9:00 a.m.		125	
4:00 p.m.		114	
April 8			
9:00 a.m.		105	Millet and cabbage
April 7	Rabbit II*		
8:45 a.m.	Control	125	
9:00 a.m.	20 cc. raw turnip juice		
9:45 a.m.		167	
11:00 a.m.		125	
12:00 noon		118	
2:00 p.m.		125	
4:00 p.m.		114	
April 8			
9:00 a.m.		118	
April 8	Rabbit III*		
9:15 a.m.	Control	100	
9:25 a.m.	20 cc. raw turnip juice		
9:55 a.m.		133	
11:35 a.m.		130	
2:00 p.m.		500	
3:35 p.m.		91	

* Starved for 24 hours before the first portion of blood was taken and during the whole period of the experiment.

TABLE 2

Effect of subcutaneous injections of turnip juice on blood sugar and phosphorus

TIME	PROCEDURE	SUGAR	INORGANIC PHOSPHORUS	DIET
		mgm. per cent	*mgm. per cent*	
April 9	Rabbit IV*			
8:45 a.m.	Control	117	5.53	
9:00 a.m.	20 cc. raw turnip juice			
10:00 a.m.		133	5.23	
1:30 p.m.		133	4.76	
April 12	Rabbit V*			
9:25 a.m.	Control	105	4.17	
9:30 a.m.	20 cc. raw turnip juice			
10:30 a.m.		111	4.35	
12:00 noon		118	4.17	
2:00 p.m.		162	3.77	
April 13	Rabbit VI*			
8:55 a.m.	Control	100	5.53	
9:05 a.m.	20 cc. raw turnip juice			
9:35 a.m.		117	4.35	
11:30 a.m.		133	4.55	
2:00 p.m.		153	4.55	Millet and cabbage
April 14	Rabbit VII*			
9:00 a.m.	Control	90	5.00	
9:40 a.m.	20 cc. boiled turnip juice			
10:30 a.m.		143	5.00	
12:00 noon		250	5.88	
2:00 p.m.		333		
April 16	Rabbit III*			
9:35 a.m.	Control	105	4.17	
9:40 a.m.	20 cc. boiled turnip juice			
12:00 noon		181	5.00	
2:00 p.m.		166	6.03	
April 19	Rabbit IV*			
9:50 a.m.	Control	100	5.53	
10:00 a.m.	20 cc. raw turnip juice with 1 cc. adrenalin (1:1000)			
12:00 noon		333	4.35	
2:00 p.m.		333	5.00	

* Starved for 24 hours before the first portion of blood was taken and during the whole period of the experiment.

TABLE 3

Effect of turnip feeding and turnip juice injections on blood cholesterol

TIME	PROCEDURE	CHOLESTEROL	DIET
		mgm. per cent	
May 4	Rabbit I*		
11:55 a.m.	Control	80	
12:00 noon	20 cc. raw turnip juice injected subcutaneously		
2:00 p.m.		76	
4:00 p.m.		73	
May 5			
9:30 a.m.		57	Millet and cabbage
May 5	Rabbit II*		
9:25 a.m.	Control	67	
9:30 a.m.	20 cc. raw turnip juice injected subcutaneously		
12:00 noon		56	
3:00 p.m.		52	
November 1	Rabbit VIII*		
9:30 a.m.	Controls	141	Millet and cabbage
2:00 p.m.		138	
November 4			
9:30 a.m.		141	Raw turnip (from November 1)
November 5			
9:30 a.m.		114	
November 8			
9:40 a.m.		83	Millet and cabbage (from November 5)
October 31	Rabbit IX*		
9:30 a.m.	Control	77	
November 1			Millet and cabbage
9:40 a.m.	Controls	80	
2:10 p.m.		80	
November 4			
9:40 a.m.		32	Raw turnip (from November 1)
November 5			
9:40 a.m.		57	
November 8			
9:50 a.m.		77	Millet and cabbage (from November 5)
October 31	Rabbit X*		
9:40 a.m.	Control	72	
November 2			Millet and cabbage
10:00 a.m.	Control	67	
November 4			
9:50 a.m.		46	Raw turnip (from November 2)
November 5			
9:50 a.m.		68	

* Without starvation

spread. Dobreff (7) showed that injections of secretin, obtained from spinach or Urtica, produce hyperglycemia in normal pigeons and dogs. According to Bickel (3), (4) secretins are thermostable. The secretin of spinach is active even after boiling at 100°C. for 24 hours. In the present experiments, boiled as well as raw turnip juice was found to produce hyperglycemia,[2] resembling the vegetable secretins also in this point.

Phosphates. Raw turnip juice produces a drop in inorganic phosphates of the blood which persists for at least five hours. Boiled turnip juice is followed by a rise. The close resemblance of the hyperglycemic effect of secretin with that of adrenalin (6), (14) may lead one to suppose that a similar relationship exists in their behavior toward the inorganic phosphates. According to Woringer (19), adrenalin given subcutaneously produces a fall of the inorganic phosphates, followed by a slight rise. Vollmer's (18) observation on man confirms this. Our experiment of April 13 (rabbit VI) shows a diphasic curve in the phosphates. The experiment of April 19 (rabbit IV) shows that, injected simultaneously, the turnip secretin and adrenalin produce a marked rise in the blood sugar with a diphasic curve for inorganic phosphorus, which is typical of adrenalin.

Cholesterol. According to Baumann and Holly (2), the average cholesterol content of normal rabbit blood is about one-half of that of human blood. Horvath (10) showed that the fluctuation of the blood cholesterol in normal rabbits ranges from 0 to 14 mgm. per cent in a period of 5 days. The controls represented in table 3 of the present paper shows that the fluctuation of the blood cholesterol in rabbits is close to the limit of technical error. We may therefore say that the cholesterol content in the blood of rabbit is fairly constant on a constant diet.

Feeding raw turnip as a single food resulted in a marked drop of the blood cholesterol. Such an effect cannot be attributed to a low cholesterol content of the turnip because the blood cholesterol of rabbits IX and X showed on the same turnip diet a drop followed by a rise. Besides, it has to be taken into consideration that subcutaneous injections of raw turnip juice produce also a drop in the blood cholesterol while the rabbits are fed on a diet of millet and cabbage (rabbits I and II, experiments of May 4 and 5).

The experiment with rabbits IX and X showed that continuous feeding of raw turnip does not keep the blood cholesterol at a very low level, showing in that an analogy with the action of insulin (13). The data obtained on rabbit VIII shows that an abnormally high blood cholesterol may be restored to normal by feeding turnip.

At present nothing definite can be said of the mechanism by which turnip

[2] Boiling did not change the amount of copper-reducing substances present in the turnip juice.

juice lowers blood cholesterol. According to van Noorden and Salomon (15) the cells of the liver are the natural and most important site of excretion of cholesterol, which seems to be partly converted there into a cholic acid-sulphur compound, taurin. *Raphanus sativus* is considered to be rich in sulphur. According to Sherman (16), the turnip contains 0.065 per cent of this element. König (12) states that the turnip contains the allyl and butyl oils of mustard.

The capacity of the turnip to affect the metabolism of cholesterol may throw some light on the curative effect of the turnip juice in cholelithiasis. Grumme (9) supposed that in cholelithiasis turnip juice might also stop the inflammation in the bile bladder. This point of view is supported by our experiments on pigeons (not presented yet) in which raw turnip juice given per os produced a marked improvement in enteritis.

SUMMARY

1. Turnip juice contains a thermostable substance which, injected subcutaneously, produces hyperglycemia in rabbits.

2. Subcutaneous injections in rabbits of raw turnip juice are followed by a decrease in the inorganic blood phosphates, while boiled turnip juice results in a rise.

3. Subcutaneous injections of raw turnip juice or the feeding of raw turnips produce a fall in the cholesterol content of the blood.

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THE REGULATION OF RESPIRATION

VIII. The pH of the Arterial Blood and Respiration Volume as Affected by Blood Volume Changes

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For many years it has been the general belief that the hydrogen ion concentration of the arterial blood is extremely constant, varying normally only within very narrow limits. This has grown out of a great number of observations of many workers. Haldane and Priestley (1905) showed the sensitivity of the respiratory center to minute changes in carbon dioxide tension of the alveolar air. After the perfusion experiments of Winterstein and the work of Hasselbalch, this was interpreted by Haldane (1922) as attempts of the respiratory center to keep the arterial hydrogen ion concentration constant. The variation he believed to be so minute that present physical and chemical methods are too crude to detect it.

That wide variations do occur in the reaction of the blood has been extensively shown and reported recently. Barr (1923) studied the effect of severe muscular exercise and found that a fall in pH of as much as 0.19 may result. Haldane, Kellas and Kennaway (1919–1920) report a constant alkalemia while living at high altitudes. Henderson and Haggard (1920), too, find that low oxygen pressures will result in an increase in the arterial pH. The effect of various anesthetics was studied by Van Slyke, Austin and Cullen (1922) and also by Leake, Leake and Koehler (1923). Both groups report an acidemia. Fraser, Ross and Dreyer (1922) found an alkalemia in cardiac dyspnea, while a relative acidemia accompanied dyspnea of severe bronchitis.

The state of the respiration was extremely inconstant under the several conditions reported by the above workers, but nowhere did they correspond to the estimated changes according to Haldane (1922). The theory of Gesell (1922) relating respiratory volume to the acidity of the respiratory center itself appears to harmonize the conflicting data. According to this theory the acid relations are not determined solely by the hydrogen ion concentration of the arterial blood but by the metabolism of the respiratory center itself and the effectiveness of the blood stream in carrying away the acid products of the metabolism.

The transport of blood gases was shown to be markedly affected by changing the volume-flow of blood. Haggard and Henderson (1922) reported lowered alveolar carbon dioxide after hemorrhage accompanied by a lowered arterial carbon dioxide. Hastings, Coombs and Pike (1921) varied the volume-flow to the brain by compressing the carotid and vertebral arteries. They obtained increased respiration and lowered arterial carbon dioxide. We have in this study reduced the volume-flow by hemorrhage and increased it by replacement with gum acacia-saline solutions.

METHOD. Dogs decerebrated by the method described by Schmidt (1923) were used for the first series. Chloroform and ether mixture was used to induce anesthesia, it was continued by ether during the decerebration operation which required fifteen to twenty minutes. The animal was placed on electric heating pads regulated to 37.5°C. by a rectal thermostat. Forty-five minutes to an hour was allowed for the ether to blow off. The animal was then connected by tracheal cannula to a rebreathing device of 99 liters capacity. A soda lime cartridge removed the carbon dioxide from the expired air, and oxygen was replenished at frequent intervals. Occasional analyses of the air in the tank were made.

In the second series of experiments morphine-urethane anesthesia was substituted for the decerebration. To prevent, as far as possible, depression of the respiratory center the morphine was reduced to a minimum, 3 mgm. per kilogram body weight. Urethane was given per rectum fifteen minutes after the morphine, 0.5 gram per kilogram body weight. Occasionally this had to be reinforced during the experiment.

Blood pressure, respiration and time in seconds were recorded on smoked paper. Samples of blood were drawn under oil from the right carotid artery and the pH determined for the whole blood by the method of Hastings and Sendroy (1924).

After the animal had been allowed to breathe from the tank for several minutes two blood samples were taken at one-minute intervals. The animal was then bled from the right carotid. The hemorrhages varied from 0.25 per cent to 1.0 per cent of the body weight. One minute after bleeding a sample was taken. The hemorrhages were continued until about 2.5 per cent to 3.0 per cent had been taken. Gum-saline (6 per cent gum acacia in 0.9 per cent sodium chloride) at 38°C. was injected rapidly into the femoral vein in several stages. Blood samples were taken frequently. Further hemorrhages and injections were continued until the animal died in some cases.

Beginning with experiment 9 the gum-saline was adjusted to pH 7.40 with sodium hydroxide, since it was found that the plain solution was quite acid, between pH 4.0 and 4.5. This was found to be an unnecessary precaution. On account of the great buffering power of the blood the effects of injection of adjusted and unadjusted gum-saline were the same.

When duplicate samples were taken the widest variation found was 0.03 pH. The readings were checked by one or more observers so that we believe our limit of error is about 0.03 pH. Since in nearly all cases the changes are far greater than this we feel the method is sufficiently accurate for the purpose.[1]

In the first series twelve animals were used. No results were obtained from one due to faulty indicator solution. In two others the animals showed an irritative breathing—shallow, rapid and irregular, so that measurements were impractical. Of the nine two were control experiments during which there was no loss of blood except for the pH samples (about 2 cc. for each sample).

Results. Figure 1 represents the results obtained in two control experiments in which no blood was drawn except for samples. A was from a decerebrated animal; in B the animal was anesthetized with morphine and urethane. In A and B pH and ventilation are fairly constant. There was some slight circulatory change, which indicates the changes that might be expected without gross changes in blood volume.

In figure 2 we have the results of three of the experiments on decerebrated animals in which the volume-flow of blood was varied by hemorrhage and injection. In A the parallelism of pulmonary ventilation and blood pH is quite marked. In B and C the pH changes are of greater degree. Heart rate and blood pressure are also more variable. In B ventilation began to change before arterial pH; in C after the change in pH.

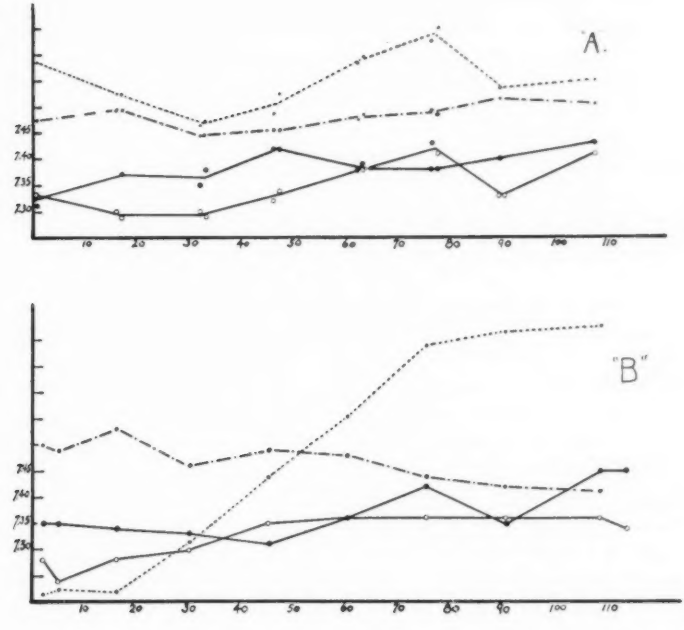
Figure 3 represents three of the experiments done on animals anesthetized with morphine and urethane. In all cases the variations were of lesser degree than when decerebrated animals were used. It will also be noticed that the blood is less alkaline.

Figure 4 represents the results obtained in an experiment planned as a control but in which traumatic shock developed. The results are directly parallel to those in which the blood volume was altered by hemorrhage. This shows the similarity between these two conditions (Gesell, 1919).

Discussion. The various changes described in the two series of experiments have been of the same nature. Those of the second, however, are somewhat blanketed by the anesthesia. In general we have obtained an increase in arterial pH on hemorrhage.

The changes in pH were very rapid. In experiment 2 there was an in-

[1] Since writing this paper M. A. Bennett, Journ. Biol. Chem., 1926, lxix, 697, has called attention to variations in the correction necessary to convert the colorimetric reading at 20° C. to the electrometric pH at 38°C. over long periods of time following hemorrhage. But judging from table 1 of her paper her findings do not materially affect results of the acute experiment of short duration. The results of our experiment are in agreement with the data obtained under similar conditions with the manganese dioxide, quinhydrone and hydrogen electrodes, Gesell and Hertzman, This Journal, Proc. Amer. Physiol. Soc., 1926, lxxviii, 206.



Key to figures

●———● pH
○———○ Respiratory minute volume
●—·—● Blood pressure
●- - - -● Heart rate
↑ 50 cc. injection of gum saline
50
↓ 50 cc. hemorrhage
50

Fig. 1. Control experiments showing the constancy of pH and respiration in the absence of hemorrhage.

A. Dog 13.5 kgm.—decerebrated. Arterial pH remained quite constant with some tendency to increase—the total change during the 107 minutes of the experiment being 0.11. Respiration shows the same constancy, increasing 1.2 liters per minute. Heart rate shows considerable variance, total 35 per minute. Blood pressure changed only 14 mm. Hg.

B. Dog 7.6 kgm.—anesthetized with morphine and urethane. The arterial pH fell slightly, 0.04 during the first 60 minutes, then remained the same. The heart rate rose after 16 minutes from the low rate of 56. Compare the heart records of the other anesthetized dogs.

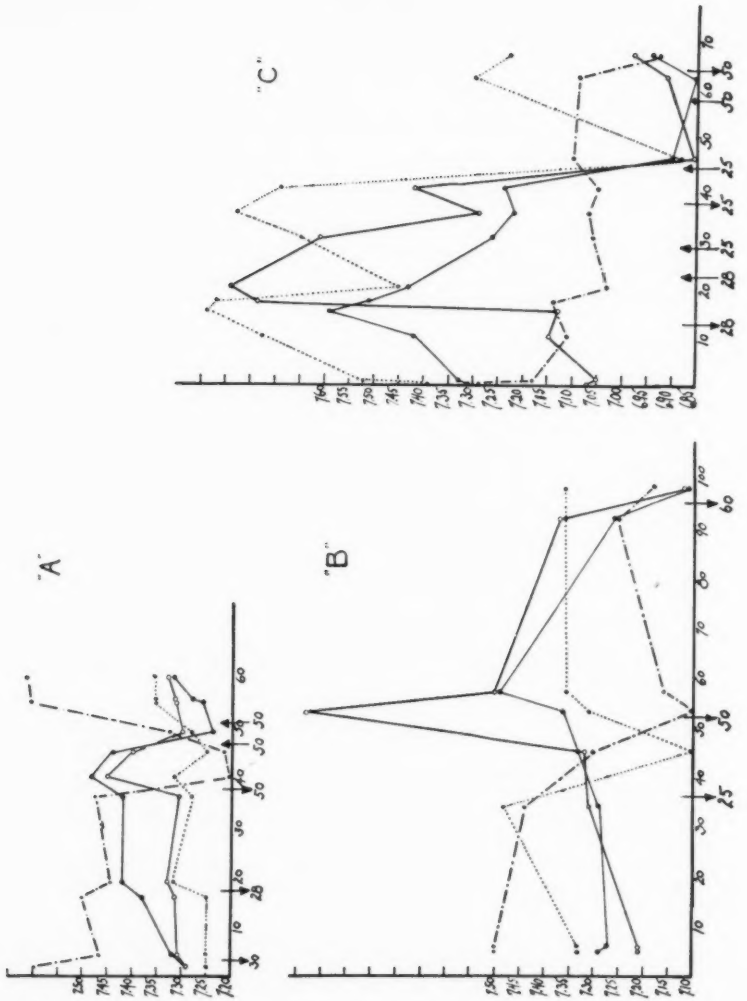


Fig. 2. Showing the effect of hemorrhage on the arterial pH and respiration of decerebrated dogs.

A. Dog 5.3 kgm. There is unusual parallelism shown between the four curves. Each hemorrhage produced increases in pH, respiration and heart rate, and a drop in blood pressure. The first injection produced a fall in pH from the high level to 0.06 below the normal. In spite of the second injection there was a rise during the following ten minutes to 0.03 above the normal. Final conditions corresponded to those at the beginning of the experiment.

crease of 0.11 in four minutes; in 9 a fall of 0.20 in eight minutes; in 11 a rise of 0.12 in nine minutes and a fall of 0.25 in eight minutes. In number 10, following hemorrhage, there was recorded a rise of 0.17 in five minutes; during the following five minutes a fall of 0.16. These changes cannot in any degree be attributed to the solutions injected per se since no difference could be noted when the acid of gum was neutralized and the pH raised to 7.4.

As rapid and as extensive as these changes may seem we cannot say that we have detected their full extent. At best the method is "spotty;" one has no means of determining what is going on between samples. Despite these disadvantages it does show extremely significant variations in arterial pH.

The exceptions to the general directional change have been of two kinds: in the first series after 2 per cent or more of the body weight had been taken, further hemorrhage resulted in decreased pH with either increased or decreased respiration, usually the former. The critical point had been passed and the animal declined rapidly. Large injections of gum-saline were not effective in staying the quick approach of death.

The other exception was seen in the first part of the experiments of the second series. This we believe to be due to too great depression from anesthesia. After some blood had been drawn and replaced by gum-saline the reaction to further hemorrhage and injection was similar to that obtained in the first series.

This depression can probably be explained by the findings of Alexander and Cserna (1925). During anesthesia with ether, oxygen consumption by the brain was reduced 79 per cent. If the metabolism of the respiratory center is depressed changes in the effective flow through the center will have less effect upon it.

In nearly every experiment one of the earlier hemorrhages produced an increase in pH without an increase in respiration. This rise in pH may be explained then by the slower flow through the lungs so that the blood is in relation with the alveolar air for a longer time. It is evident that the pH change was greater where respiration increased than where there was no

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B. Dog 10.2 kgm. The first hemorrhage, 25 cc., produced slight effect on pH and respiration. The second, 50 cc., increased respiration nearly threefold, but the total effect did not last; pH was slower in responding, rising slowly at first, then more markedly. During an interval of 35 minutes there was a tendency for return to normal. The next large hemorrhage, 60 cc., produced decline from which there was no recovery.

C. Dog 5 kgm. The changes in this experiment were quite marked. Total pH variation was from 7.58 to 6.85. Hemorrhage and injection of gum-saline were alternated. Response was noted first in pH; respiration showed a larger change delayed at first then the courses paralleled. Blood pressure was low throughout—88 mm. Hg being the highest point with an average of about 50 mm.

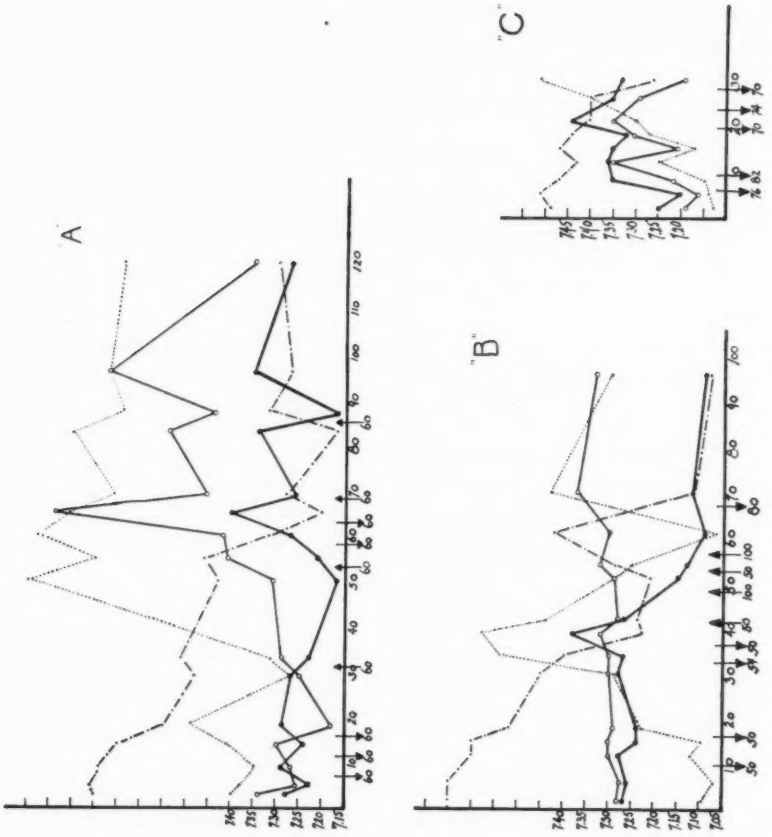

Fig. 3. Showing the effect of hemorrhage on the arterial pH and respiration of dogs anesthetized with morphine and urethane.

A. Dog 12.4 kgm. During the first 50 minutes the results were very irregular. The heart was markedly slowed by the morphine. The heart rate rose after three 60-cc. hemorrhages and two 60 cc. injections. Thereafter the response to hemorrhage and injection was regular and pH and respiration ran parallel courses.

B. Dog 9.5 kgm. Response of arterial pH and respiration were very slight throughout, and increased, however, after three 50-cc. hemorrhages. The heart was slow at the start, later increasing in rate. Blood pressure responded to blood volume changes.

C. Dog 15.4 kgm. Five hemorrhages were done in this experiment. No saline was injected. Each of the first three, totalling 1.5 per cent body weight, was followed by increase in pH and respiration; the last two by decrease. The heart was slow at first but increased rapidly.

increase. The more rapid ventilation lowered the carbon dioxide tension of the alveoli and consequently the arterial blood.

These results are entirely in accord with the view held by Gesell (1923). Hemorrhage reduces the volume-flow of blood resulting in a reduction of the amount of oxygen delivered to the tissues, and consequently in the supply of carriers for carbon dioxide from the tissues. This backing up causes the center to become less alkaline. The resulting increase in respiration lowers the alveolar carbon dioxide tension, and increases the alkalinity of the blood.

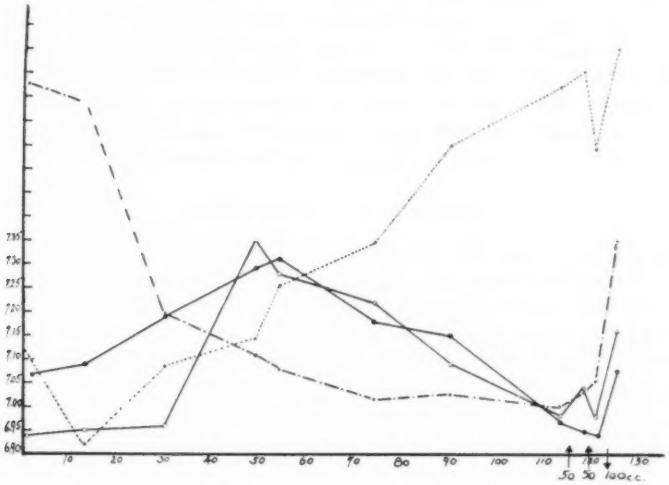

Fig. 4. Showing the effect of traumatic shock on arterial pH and respiration on a decerebrate animal, 10.4 kgm. No hemorrhages were done during this experiment. The blood pressure high (185 mm. Hg) at the outset fell rapidly to 53 mm. Hg. The heart rate increased from 114 to 270 per minute. During the fall of blood pressure respiration and pH rose, then fell with parallel courses. Attempts to raise the blood pressure by gum-saline injections were of no avail although slight temporary recovery occurred.

The injections with gum-saline solution, although made slightly more alkaline than the blood, resulted in an increase in the hydrogen ion concentration. Decrease in respiration volume usually accompanied this increase in volume-flow of blood.

The erratic results obtained after considerable hemorrhage are probably due to the poor condition of the respiratory center. The state produced by the hemorrhage is, as we have pointed out before, analogous to low oxygen tension in the inspired air. Loevenhart (1915) states, "The effect of re-

ducing oxidation in these centers depends on three factors, first on the extent to which the oxidative processes are reduced; second, the suddenness with which they are reduced; third, the condition of the centers."

Roberts (1924) observed the effect of reducing the volume-flow to the brain of cats and rabbits. He found that apnea followed occlusion of the carotids. In a considerable number of observations on dogs we have noted all three of the possibilities: increased, decreased and unchanged respiration. An increase was by far the most frequent. Explanation of the other responses can be found in the statement of Loevenhart just quoted.

If the erratic results obtained after considerable hemorrhage are due to an impaired condition of the respiratory center following impaired oxidations they support the view of the importance of acidity as a factor controlling respiration. On the other hand—insofar as increased acidity of the respiratory center and decreased oxidation develop simultaneously in hemorrhage, the results of these experiments do not preclude a direct stimulating effect of lack of oxygen when the usual effects are obtained.

SUMMARY AND CONCLUSIONS

The pH of the arterial blood and minute volume of respiration of dogs were studied simultaneously as affected by hemorrhage and subsequent injection with gum acacia-saline solution.

Two series of experiments were run: one in which anesthesia was produced by decerebration, the second by morphine and urethane.

Hemorrhage produced increased pH of the arterial blood and usually an increase in respiration. Injection gave the opposite results. The results of the second series were similar to the first, though less marked.

The change in pH we believe to be due to two main factors: 1, change in the minute volume of air respired, and 2, change in the volume-flow of blood through the lungs.

The pH of the arterial blood has not been shown to change the respiratory volume but rather the respiratory volume has tended to change the arterial pH.

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OBSERVATIONS ON THE FUNCTION OF THE FROG'S KIDNEY[1]

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Because of its curious blood supply the kidney of the frog has been used in a number of investigations of renal function, and conclusions drawn from such experiments have been applied to the mammalian kidney. Most of these experiments have been carried out under artificial conditions, and have thrown little light on the normal physiology of the frog's kidney. That it does not develop into a metanephros but persists as a mesonephros suggests that its function may not be entirely analogous to that of the mammalian kidney.

The anatomical differences most suggestive of functional differences are the double blood supply to the uriniferous tubules of the frog's kidney, and the absence of a loop of Henle between the proximal and distal convolutions. Four distinctive regions possessing characteristic epithelium have been described, however, as in the mammalian kidney (1).

Such work as has been done on the normal function of the frog's kidney has been handicapped by the small amounts of blood and urine obtainable from small frogs. Observations have been made by collecting composite samples from a number of animals, or by collecting the urine over long periods. By these methods no accurate comparison of blood and urine is possible, particularly when studying the effects of varying conditions.

Toda and Taguchi (2) collected the urine of summer frogs by tying off the cloaca and allowing the urine to collect in the bladder for twenty-four hours. They describe the urine as a waterclear, almost colorless to pale yellow fluid, usually faintly acid to litmus, occasionally neutral or alkaline. The specific gravity is between 1.0009 and 1.0018, the osmotic pressure 1.08 to 1.44 atmospheres ($\Delta = 0.085 - 0.120$). Total solids average 0.246 per cent, organic matter 0.193 per cent, and ash 0.053 per cent.

Van der Heyde (3) analyzed the water in which ten to fifteen frogs had been kept twenty-four hours. In these composite samples he found the

[1] The observations recorded in this paper were presented before the American Physiological Society, December, 1924 (This Journal, 1925, lxxii, 189).

following average values for the non-protein nitrogenous constituents of blood and urine:

	TOTAL N	UREA N	AMMONIA N	URIC ACID N
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
Urine	20.6	18.0	1.05	0.058
Blood	17.3	16.3		0.43

The most complete study of the concentrating power of the frog's kidney has been made by St. V. Przylecki. Unfortunately his report of his experiments, which is published in Polish, is not accessible, but the results are quoted by Parnas (4) in whose laboratory the work was done.

Przylecki increased the salt content of the frog's blood by injection of sodium sulphate or sodium chloride, or by placing the animals in solutions of these substances, and by determining the freezing point of the blood and urine observed the effect upon their osmotic pressure. He found that when the frog was in water the salt was simply excreted in the form of a very large amount of dilute urine. If the frog was dry, the urine concentration increased with that of the blood, gradually approaching it. The greater the concentration of the salt in blood and urine, the smaller the urine quantity became, and finally anuria and death resulted before the urine concentration equalled that of the blood. Przylecki concludes that under conditions which cause the warm-blooded animal to excrete a urine more concentrated than his internal fluids the frog excretes the disturbing excess in the form of a hypotonic solution, or else he cannot excrete it. The frog's kidney can excrete only a urine of lower molecular concentration than that of the blood flowing through the kidney.

On determining the urinary N and the rest N of the blood, Przylecki found that nitrogen is always more concentrated in the urine, so that the urine is always poorer in salts and richer in N than the blood.

From his results Przylecki concludes, with Bainbridge, Menzies and Collins (5), that in the glomerulus is filtered a fluid isotonic with plasma, and from this a hypertonic salt solution is reabsorbed in the tubules; this salt solution may also contain sugar, etc. In this manner there remains a urine hypotonic in salts, enriched in excretory products.

In the series of experiments to be reported here, it was possible to make a more direct and complete investigation of the physiology of the frog's kidney than has previously been reported because of the large size of the frogs studied. They are of the species Rana catesbiana, and they vary in weight from four or five hundred grams to nine hundred or even a thousand grams.

With these large animals which make it possible to collect urine over short periods and to compare it with blood drawn at the same time, it was felt to be worth while to study more thoroughly the normal function of the frog's kidney.

METHODS. Urine was obtained from the bladder very simply by catheterization, and as the frogs were found to empty the bladder relatively infrequently no precautions were necessary to prevent loss of urine during the periods studied. Only very rarely was there contamination with feces. Blood samples were drawn from the heart by means of a syringe and needle inserted through the skin. The experiments were performed without anesthesia or any operative procedure, so that experimental conditions were kept entirely normal.

The studies were made during the winter and spring months. A fresh supply of frogs was received about once every six weeks. They were kept in large tanks of running water, and when brought to the laboratory were placed in wire cages set in pans of water. They received no food for at least a week and usually longer before the experiment.

Urine collections were made over comparatively short periods, the length of the period depending upon the degree of diuresis, and the amount of urine required for analysis. The withdrawal of 1 or 2 cc. of blood apparently caused no injury to the frog or demonstrable change in the concentration of the blood or urinary constituents, but to avoid errors from this source there was usually a blood sample drawn at the end of the period of urine collection, or two urine samples were collected, one before and one after the blood was drawn. Coagulation was prevented by powdered potassium oxalate, except in a few experiments with phenolsulphonephthalein, in which the blood was allowed to clot and the serum analyzed.

To induce diuresis distilled water was injected into a lymph sac. Subcutaneous or intravenous injections were used to introduce substances into the blood stream, in other experiments the frogs were placed in solutions of the substance and allowed to absorb it through the skin.

Urea determinations were made by means of urease, aeration and titration, using 0.01 N acid, 0.02 N alkali and micro-burettes. Bicarbonates were determined by means of the Van Slyke-Stadie apparatus (6), phosphates by Briggs' modification of the Bell-Doisy method (7), and chlorides by the micro-method of Van Slyke (8). Benedict's method (9) was used for uric acid determination. Phenolsulphonephthalein concentrations were determined colorimetrically.

RESULTS. A comparison of the concentration of the chief urinary constituents as found in normal blood and urine is presented in table 1. This shows that normally only minute amounts of chloride and bicarbonate are present in the urine; much less than the concentration in the blood. Phosphates are also present only in small amounts, the concentration may be slightly greater than that in the blood, though it is usually less. Very little uric acid is found in blood or urine, it may be somewhat more concentrated in the latter. In contrast to the above substances, which normally are very slightly concentrated by the kidney, if at all, urea is very

definitely concentrated, and values as high as nineteen times that in the blood have been found for urine urea under perfectly normal conditions. Most frequently the urea in the urine is six to eight times as concentrated as that in the blood. The blood urea values are surprisingly low, in two frogs concentrations of 24 mgm. per cent and 30 mgm. per cent were observed, and in one the concentration was only 3.8 mgm. per cent, but usually the values for normal blood urea are found between 7 and 10 mgm. per cent.

In order to determine to what degree the frog's kidney can concentrate the substances which it eliminates, the effect of increasing the concentra-

TABLE 1

Typical values for normal concentration of chief urinary constituents in blood and urine

	SUBSTANCE	
	Blood	Urine
	mgm. per cent	*mgm. per cent*
Chloride (Cl)	168.0	5.0
	222.0	0.0
	180.0	14.0
Bicarbonate (HCO_3)	196.0	25.0
	158.0	30.0
	193.0	22.0
Phosphate (PO_4)	21.1	10.4
	23.6	27.6
	15.6	<2.0
Urea	8.0	49.0
	8.6	164.0
	7.0	59.0
	30.0	54.0
	3.8	23.0
Uric acid	0.8	1.2

tion of these substances in the blood was determined. The effect of increasing blood chloride was first studied. If the frog is in water, a diuresis results when the chloride content of the blood is increased, but when the concentration of the salt in the blood reaches a value above about 225 mgm. per cent Cl, the concentration in the urine begins to rise. As the amount in the blood is increased, that in the urine approaches it, but it is not until the blood value is so high as to cause definite symptoms of intoxication that the curves cross, and the urine concentration is never more than a fraction above that in the blood. In several of the experiments in which this point was reached, simultaneous determination of carbonates showed

that the molecular concentration of the two salts was never higher in the urine than in the blood.

The following two protocols are typical of eight experiments showing similar results:

Protocol I. January, 1923.		Frog D. Weight 537 grams	
1/11, 1/12, 1/13		Injections into lymph sac of 0.7% NaCl	
1/14 p.m.	3:30	Inj. 105 cc. 0.7% NaCl. Drying	
1/15 a.m.	10:00	No urine, eyes clouded. Placed in water	
	10:00–12:30	1 cc. urine	Cl 266 mgm.%
p.m.	12:30– 4:30	21 cc. urine	Cl 163 mgm.%
	5:00	Inj. 90 cc. 0.7% NaCl	
1/16 a.m.	10:00	Slow drying	
	10:00–11:10	3.5 cc. urine	Cl 290 mgm.%
	11:30	3.0 cc. blood	Cl 398 mgm.%
	11:10–12:20	0.8 cc. urine	Cl 344 mgm.%
p.m.	4:00	Inj. 110 cc. 0.7% NaCl	
1/17 a.m.	?–10:00	80.0 cc. urine	Cl 482 mgm.%
	10:00–11:00	0.3 cc. urine	Cl 390 mgm.%
	11:00	Inj. 33 cc. 1.0% NaCl	
	11:00–12:30	0.6 cc. urine	Cl 438 mgm.%
p.m.	1:00	Inj. 20 cc. 1.0% NaCl	
	12:30– 2:10	6.0 cc. urine	Cl 494 mgm.%
	2:40	1.0 cc. blood	Cl 502 mgm.%
	2:10– 2:50	0.3 cc. urine	Cl 352 mgm.%
	2:45– 2:50	Inj. into heart 10 cc. 2.0% NaCl	
	2:50– 4:10	0.3 cc. urine	Cl 553 mgm.%
	4:10	1.5 cc. blood	Cl 533 mgm.%
	4:10– 5:00	No urine. Eyes clouded	

Protocol II. February, 1924.		Frog 12. Weight 740 grams	
2/11 p.m.	2:30	Urine	Neg. for Cl
		Blood	Cl 220 mgm.%
			HCO_3 208 mgm.%
		Placed in bath of 1% NaCl	
2/12 p.m.	?–12:30	0.2 cc. urine	Cl 182 mgm.%
	2:30	Inj. 110 cc. 1% NaCl into lymph sac. Dried until 5:00, then replaced in 1% NaCl. Eyes clouding, no urine	
2/12, p.m.	5:00-2/13, p.m. 2:30	7.5 cc. urine	Cl 620 mgm.%
			HCO_3 30 mgm.%
2/13 p.m.	2:30	4.0 cc. blood	Cl 583 mgm.%
			HCO_3 104 mgm.%
	2:30–4:10	2.3 cc. urine	Cl 592 mgm.%

Experiments on the excretion of bicarbonates give results similar to those obtained with chlorides. With a rise in blood bicarbonate the urinary bicarbonate increases, but it cannot reach a concentration sufficient to give a urine of higher osmotic pressure than the blood. A concentration in the urine of 1.3 times that in the blood was the greatest obtained, and a

concentration of the salt in the blood high enough to cause the excretion of a urine of the same concentration was always toxic to the frog, as evidenced by muscular twitching and death if the condition was not relieved.

Two protocols are given as representative of five similar experiments.

Protocol III. March, 1924. Frog 26. Weight 675 grams

3/3	a.m. 11:30–2:00	1 cc. urine	HCO_3 24.5	mgm.%
	p.m. 2:00	Inj. 60 cc. 1% $NaHCO_3$ into lymph sac		
	4:45	1.5 cc. blood	HCO_3 294	mgm.%
	4:43–5:20	1.6 cc. urine	HCO_3 130	mgm.%
	6:00	Inj. 45 cc. 1% $NaHCO_3$		
3/4	a.m. 10:15	Inj 50 cc. 1% $NaHCO_3$		
	p.m. 1:00–2:00	4.5 cc. urine	HCO_3 455	mgm.%
	2:00–3:05	13.0 cc. urine	HCO_3 398	mgm.%
	3:10	1.0 cc. blood	HCO_3 348	mgm.%
	3:05–4:00	6.0 cc. urine	HCO_3 322	mgm.%
		In bath of 0.75% $NaHCO_3$		
3/5	a.m. 10:15	Inj. 50 cc. 1% $NaHCO_3$		
	p.m. 2:45	Inj. 50 cc. 1% $NaHCO_3$		
3/6	a.m. 10:30	Inj. 50 cc. 1.5% $NaHCO_3$		
	p.m. 12:40	Inj. 50 cc. 1.5% $NaHCO_3$		
	2:00–2:30	2 cc. urine	HCO_3 700	mgm.%
	2:33	2 cc. blood	HCO_3 533	mgm.%
		Marked intoxication		
	2:30–3:00	0.2 cc. urine	HCO_3 670	mgm.%

Protocol IV. March, 1924. Frog 24. Weight 760 grams

2/27		Normal urine	HCO_3 22	mgm.%
			Cl 1	mgm.%
		Normal blood	HCO_3 194	mgm.%
			Cl 209	mgm.%
3/3		Inj. 135 cc. 1% $NaHCO_3$ into lymph sac		
3/4	a.m. 10:10	Inj. 50 cc. 1% $NaHCO_3$ into lymph sac		
	p.m. 1:00–2:00	9.5 cc. urine	HCO_3 430	mgm.%
	2:00–2:55	12.0 cc. urine	HCO_3 354	mgm.%
	2:55	1.5 cc. blood	HCO_3 389	mgm.%
	2:55–4:00	6.0 cc. urine	HCO_3 215	mgm.%
		Placed in bath of 0.75% $NaHCO_3$		
3/5	a.m. 10:00	Inj. 50 cc. 1% $NaHCO_3$		
	p.m. 2:45	Inj. 50 cc. 1% $NaHCO_3$		
	5:00	Inj. 50 cc. 1% $NaHCO_3$		
3/6	a.m. 10:30	Inj. 50 cc. 1.5% $NaHCO_3$		
	p.m. 12:40	Inj. 60 cc. 1.5% $NaHCO_3$		
	2:00–2:25	2.5 cc. urine	HCO_3 655	mgm.%
			Cl 4	mgm.%
	2:25	3.0 cc. blood	HCO_3 576	mgm.%
			Cl 107	mgm.%
		Marked intoxication		
	2:25–2:50	2.1 cc. urine	HCO_3 576	mgm.%
			Cl 7	mgm.%

The concentration of phosphates rises in the urine when it is increased in the blood, but the curve is not so smooth as that observed with chlorides or carbonates, and other factors beside blood concentration seem to affect the excretion. The most obvious of these is the degree of diuresis, for phosphates are too toxic to be pushed to a point where they might cause a decrease in water excretion. The concentration of phosphate by the kidney is greatest when the frog is drying, and values as high as six times the blood phosphate have been obtained for the urine. Simultaneous determinations of urea show it to be concentrated 18 to 65 times in the experiments in which phosphates were concentrated 4 to 6 times. The concentration of phosphate in the blood seems to have comparatively little effect on the degree to which it is concentrated by the kidney.

The three protocols given below are typical of ten experiments on phosphate excretion.

Protocol V. February, 1924. Frog 21. Weight 750 grams

2/18 a.m.	11:00	Normal urine	PO_4 10.4	mgm.%
		Normal blood	PO_4 21.0	mgm.%
p.m.	1:00	Inj. 40 cc. M/15 Na_2HPO_4 and NaH_2PO_4 (pH approximately 7.0) into lymph sac		
	2:00–5:00	1 cc. urine	PO_4 520	mgm.%
	5:00	2 cc. blood	PO_4 104	mgm.%
2/19 p.m.	1:30	Inj. 40 cc. M/15 phosphate mixture		
	2:40–4:00	10 cc. urine	PO_4 180	mgm.%
	4:05	2 cc. blood	PO_4 110	mgm.%
2/20 a.m.	9:00	Some muscular twitching		
	10:15	Inj. 60 cc. M/15 phosphate mixture		
p.m.	2:00	Marked muscular twitching		
	12:40–2:10	4.5 cc. urine	PO_4 282	mgm.%
	2:10	1.5 cc. blood	PO_4 171	mgm.%

Protocol VI. May, 1924. Frog 53. Weight 980 grams

5/1		Placed in dry cage. Slow drying		
5/2 a.m.	10:10-2:10	0.9 cc. urine	PO_4 104	mgm.%
			Urea 574	mgm.%
p.m.	2:10	2.3 cc. blood	PO_4 231	mgm.%
			Urea 8.9	mgm.%
	2:10–5:50	0.1 cc. urine		

Protocol VII. April, 1925, Frog 73.

4/7 p.m.	4:00	Bladder emptied. Frog placed in dry cage		
4/7, p.m.	4:00-4/8, a.m. 10:30	1.5 cc. urine	PO_4 46	mgm.%
			Urea* 786	mgm.%
4/8 a.m.	10:30	1.5 cc. blood	PO_4 8.5	mgm.%
			Urea* 18	mgm.%

* Ammonia was not determined in this experiment, so the figure for urea includes the NH_3 calculated as urea. This does not affect the blood urea value, but the corrected figure for urea in the urine would be about 50 mgm. per cent lower than that given above.

Increasing the amount of urea in the blood causes the urine value for

this substance to approach that of the blood, but in this case the ratio $\frac{\text{concentration in urine}}{\text{concentration in blood}}$ is decreased rather than increased as it is with chlorides and carbonates. The most efficient concentration of urea by the kidney is obtained when the blood urea is normal and the water excretion is limited by drying the frog. Under these conditions urea in the urine has been found concentrated as much as seventy-four times the blood urea. This is in striking contrast to chlorides and bicarbonates which the frog's kidney fails to concentrate at all, and even to phosphates which are not concentrated more than one-tenth this much. Protocols VI and VII illustrate the difference in the degree of concentration of urea and of phosphates.

When phenolsulphonephthalein is present in the frog's blood stream it is eliminated in much the same manner as urea. It is concentrated more efficiently when the blood concentration is low. When the water excretion is limited by drying the frog the percentage of phenol red in the urine has been found as high as sixty-five times that in the blood serum.

The following protocols are selected as typical of nineteen experiments on urea excretion and seven on phenolsulphonephthalein excretion. Protocols VIII and XI illustrate the decrease in the ratio $\frac{\text{concentration in urine}}{\text{concentration in blood}}$ when the concentration in the blood is increased. In these experiments the water excretion is kept as nearly constant as possible by inducing a diuresis by injecting water in the lymph sacs. Protocols IX, X and XII illustrate the concentrating power of the kidney for these substances.

Protocol VIII. May, 1924. Frog 60.

In order to keep the water excretion as nearly constant as possible, diuresis was maintained throughout the experiment by water injections into the lymph sac.

5/21 p.m.	12:45	Inj. 50 cc. water (and 600 mgm. glucose)* into lymph sac			
	1:40– 2:06	4.0 cc. urine	Urea	24	mgm.%
	2:10	1.5 cc. blood	Urea	3.6	mgm.%
	2:25	Inj. 400 mgm. glucose in 4 cc. water*			
	2:52– 3:20	4.5 cc. urine	Urea	34	mgm.%
	3:22	2.5 cc. blood	Urea	3.3	mgm.%
5/23 a.m.	9:30	Inj. 100 cc. water in lymph sac			
	9:45	Inj. 2 cc. 25% urea in lymph sac			
	10:45–11:15	6 cc. urine	Urea	166	mgm.%
	11:20	1 cc. blood	Urea	117	mgm.%
	11:25	Inj. 7 cc. 25% urea in lymph sac			
p.m.	12:20–12:35	4.2 cc. urine	Urea	410	mgm.%
	12:36	1.0 cc. blood	Urea	364	mgm.%
	12:40	Inj. 14 cc. 25% urea in lymph sac			
	2:30– 2:50	5.0 cc. urine	Urea	982	mgm.%
	2:55	5.0 cc. blood	Urea	838	mgm.%

* The first two periods were also used in studying glucose excretion. The sugar injections do not affect the urea excretion.

Protocol IX. April, 1926. Frog 88. Weight 720 grams

4/14 p.m.	9:00	Bladder emptied. Frog drying		
4/15 a.m.	9:30	Urine collected: 2 cc.	Urea	650 mgm.%
		Blood	Plasma urea	10 mgm.%

Protocol X. April, 1926. Frog 89. Weight 750 grams

4/14 p.m.	9:00	Bladder emptied. Frog drying		
4/15 a.m.	9:30	Urine collected: 1 cc.	Urea	670 mgm.%
		Blood	Plasma urea	9 mgm.%

Protocol XI. May 14, 1924. Frog 56. Weight 700 grams

a.m.	7:45	Inj. 100 cc. water in lymph sac		
	9:40	Inj. 2 cc. Phenol Red and 50 cc. water in lymph sac		
	10:14–10:33	2 cc. urine	Urea	79.0 mgm.%
			Phenol Red	4.3 mgm.%
	10:37	3 cc. blood	Urea	33.0 mgm.%
			Phenol Red	1.5 mgm.%
	10:40	Inj. 2 cc. Phenol Red and 1 cc. 40% urea and 40 cc. water		
	11:11–11:23	4.3 cc. urine	Urea	85.0 mgm.%
			Phenol Red	8.4 mgm.%
	11:25	2.5 cc. blood	Urea	65.0 mgm.%
			Phenol Red	2.4 mgm.%
	11:26	Inj. 5 cc. Phenol Red and 5 cc. 40% urea and 30 cc. water		
	11:56–12:08	3.8 cc. urine	Urea	281.0 mgm.%
			Phenol Red	9.6 mgm.%
p.m.	12:11	3.0 cc. blood	Urea	259.0 mgm.%
			Phenol Red	5.1 mgm.%
	12:13	Inj. 8 cc. Phenol Red and 8 cc. 40% urea		
	12:43–12:53	1.6 cc. urine	Urea	482.0 mgm %
			Phenol Red	10.0 mgm %
	12:56	4.0 cc. blood	Urea	496.0 mgm %
			Phenol Red	7.4 mgm.%

Protocol XII. June 8, 1926. Frog 92. Weight 750 grams

a.m.	10:15	Inj. 5 cc. Phenol Red in lymph sac		
	11:50	Blood	Phenol Red	4.4 mgm.%
		Very slow drying		
	11:50– 7:50	4 cc. urine	Phenol Red	288 mgm.%
p.m.	7:50	Blood	Phenol Red	1.5 mgm.%

Some experiments have been carried out to determine whether the frog's kidney concentrates glucose, either normally or after phloridzin administration. The results obtained were not conclusive because of the difficulty in ascertaining with such small amounts whether the reducing substance measured was actually glucose. The experiments afford some evidence that glucose may be concentrated at least as efficiently as phosphates, but further work is needed to settle this point.

Discussion: The results of this series of experiments demonstrate

several characteristics of the frog's kidney. It is obvious that while urea and phenolsulphonephthalein can be very definitely concentrated, phosphates are concentrated much less efficiently, and chlorides and bicarbonates not at all. The urine is never of higher osmotic pressure than the blood. This is the most striking functional difference between the mammalian and amphibian kidneys.

Since the chief anatomical difference between the frog's kidney and that of the mammal is the absence of the loop of Henle in the former, the failure of the frog's tubule to reabsorb water against osmotic pressure suggests that it may be in the loop of Henle that such reabsorption takes place. If such is the case, the power to excrete a hypertonic urine should appear first in those animals in which a loop is first developed. Huber (10) has described a very definite loop in the bird's kidney, so according to this theory the bird should be able to excrete a urine hypertonic to the blood. d'Errico reports freezing point determinations indicating that the osmotic pressure of the chicken's urine is a little higher than that of the blood (11).

The marked difference between the concentration by the frog's kidney of urea and phenol red, and the concentration of the other urinary constituents, suggests that the two groups may be eliminated by a different mechanism. Evidence that the two former substances are secreted by the tubular epithelium of the frog's kidney has been presented in a previous communication (12). This theory was based on the following facts: 1. The concentration ratio for urea and phenol red is much greater than for other bodies. 2. Both urea and phenol red occur in a much greater concentration in the cells of the renal tubules of the frog than in the blood and other tissues (with the possible exception of the liver for urea). 3. The efficiency of the frog's kidney for eliminating urea and phenol red decreases as the plasma concentrations increase,—a fact which is interpreted as being due to the secreting cells becoming saturated with the dye.

In the last edition of his monograph, *The Secretion of the Urine* (13), Professor Cushny has suggested that our findings of a higher concentration of urea in the kidney than in the blood or urine may be due to the fact that the diuresis was insufficient to open all the glomeruli, so that a more concentrated urine excreted earlier might not all have been washed out of the tubules and collecting ducts. This explanation of our results seems improbable after a consideration of the structure of the kidney and of the degree and duration of the diuresis obtained. Using the maceration method employed by Traut (14), Stewart (15) has made a careful study of the structure of the kidney in frogs of the species used in our experiments. He finds that a number of tubules drain into each collecting duct, so that even if some of the glomeruli are not opened by the diuresis, the collecting duct will be washed out by the dilute urine from other glomeruli draining into the same duct. This does not of course eliminate

the possibility that a concentrated urine has stagnated in the tubules themselves if the glomeruli have remained closed, but that seems highly improbable in such an experiment as is reported in protocol XIII.

Protocol XIII. June, 1926. Frog 94. Weight 720 grams

6/25 a.m.	11:00	Inj. 150 cc. distilled water in lymph sac	
p.m.	1:50	Inj. 100 cc. distilled water in lymph sac	
	1:50– 2:30	21 cc. urine	Urea 8.5 mgm.%
	4:00	Inj. 100 cc. water	
	8:00	Inj. 100 cc. water	
	9:15– 9:45	17 cc. urine	Urea 9.5 mgm.%
6/26 a.m.	1:30	Inj. 100 cc. water	
	8:15– 8:45	13 cc. urine	Urea 18 mgm.%
	8:45	Inj. 100 cc. water	
	8:45–12:15	75 cc. urine	
p.m.	12:15–12:45	15 cc. urine	
	12:45	Inj. 100 cc. water	
	12:45– 1:45	25 cc. urine	Urea 11 mgm.%
	2:10	Blood	Urea 8 mgm.%
	1:45– 2:20	17 cc. urine	Urea 12 mgm.%
	2:20– 2:22	Both kidneys removed	Urea 53 mgm.%

Here we have a concentration of 53 mgm. per cent urea in the kidneys, while the highest concentration in the urine during the preceding twenty-four hours was 18 mgm. per cent. Richards and Wearn (16) found that only a part of the glomeruli might be open at one time, but they do not believe that individual glomeruli remain closed for more than a short period at a time. The blood flow is thought of as constantly shifting, so that no glomerulus is long without a supply of oxygen. But assuming that some glomeruli may remain closed for a long time and the cells receive oxygen by diffusion from neighboring vessels, it is highly improbable that enough glomeruli should remain shut down during a diuresis continuous for twenty-four hours for the concentrated urine in their tubules to explain such an accumulation of urea in the kidney as is found here. That the urea is present in the cells of the renal tubules seems to be a much more likely explanation.

The evidence seems conclusive that in addition to the filtration in the glomerulus of a fluid isotonic with plasma and the reabsorption in the tubule of a hypertonic solution, as assumed by Przylecki, there is also an active secretion of urea and phenolsulphonephthalein by the tubules of the frog's kidney. Marshall and Vickers (17) showed phenol red to be secreted by the convoluted tubules in the dog, but the data do not support the idea that urea is secreted by the mammalian tubule (12). Mayrs (18), who presents evidence that uric acid is secreted in the fowl, suggests that secretion is a primitive process existing in the kidneys of animals low on the evolutionary scale, and present only as a relic in the higher animals. The above interpretation is in accord with that hypothesis.

SUMMARY

Chlorides and bicarbonates are normally present in the frog's urine in small amounts. They are increased when the concentration in the blood rises, but they cannot be concentrated by the kidney so as to give a urine of osmotic pressure higher than that of the blood.

Phosphates are present in small quantities in the normal urine. They may be concentrated as much as six times.

Urea is normally more concentrated in the frog's urine than in the blood and when water excretion is limited it may be concentrated as much as seventy-four times. The concentration ratio is decreased when the blood urea is increased.

Phenolsulphonephthalein is excreted in the same manner as urea.

The explanation of the high urea content of the kidney as compared with other tissues as due to concentrated urea stagnating in some of the tubules rather than to accumulation of urea in the cells has been shown to be improbable.

In conclusion I wish to express my indebtedness and gratitude to Dr. E. K. Marshall, Jr., under whose guidance this research was undertaken and carried out.

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KIDNEY FUNCTION IN ADRENAL INSUFFICIENCY

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Since there is no satisfactory test for adrenal insufficiency we sought through the literature for a clue to something which might be used. The work of Marshall and Davis was very suggestive in this respect. They found that the blood urea rose to about twice normal after removal of both adrenals. It remained at this higher level until just before death, when it rose still higher. The cause of this increase appeared to be the reduction in kidney function because urea and creatinin injected into the blood were excreted very slowly.

At the same time that we have studied the blood urea we have made observations upon other constituents of the blood as possibly offering further light upon the problem.

Gradinescu and Donath have reported increased concentration of the blood.

Bierry and Malloizel observed a reduction in the blood sugar.

We have made determinations upon blood solids, muscle solids, blood sugar and, in addition, the calcium, phosphorus, creatinin and uric acid of the blood.

METHODS. We attempted to produce a *transient adrenal insufficiency* in two cats by removing one adrenal and tying all of the veins to the other except a small vessel coming from adjacent fat. In this way adrenal blood could escape only through the fat vessel or the rete to the kidney (Cow). The right adrenal was removed at the first stage and the veins were tied nine days later.

Complete adrenal insufficiency was produced by removing the adrenals in two stages extraperitoneally through the lumbar path, at intervals of two or more days. Cats were used in all but one experiment.

A large number of the animals were treated with various preparations of the adrenal cortex but a sufficient number were untreated to make certain that such treatment did not appreciably alter the results, but merely prolonged life in some instances.

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Solids. The solids in blood and skeletal muscle were determined by drying weighed quantities over H_2SO_4 to constant weight. The muscles used were from the thigh.

Urea. The usual urease aeration method was used. When 1 cc. or more of blood was available urea was determined by the macro method. However, for most of the work about 0.1 gram of blood was collected from a needle prick in the marginal ear vein and weighed in a small glass tube containing the proper amount of oxalate. These tubes were then placed in the aeration tube (20 mm. by 200 mm.) and treated with about one-fifth of the amount of reagents usually used in the macro-urease method. Special care was taken in controlling the urease and acid solutions.

Sugar. The proteins were removed from whole blood by the trichloracetic acid method. After neutralizing 1 cc. of this filtrate with sodium carbonate, sugar was estimated by the ferricyanide method. Controls were run on the ferricyanide solution and sugar standard.

Calcium. Calcium was precipitated as oxalate in an aliquot part of the trichloracetic acid filtrate. The oxalate was titrated in a warm sulfuric acid solution by 0.01 N potassium permanganate. The whole operation was carried out in a 15 cc. centrifuge tube.

Phosphorus. Another portion of the above filtrate was used for an acid soluble phosphate determination by the ammonium molybdate method.

Creatinin and uric acid. Creatinin and uric acid were estimated in parts of the trichloracetic filtrate by slight modifications of the standard colorimetric methods.

Post-mortem examination. Autopsies have been performed in most instances within a short time after death. In some cases, however, the cats died during the night and the organs were examined the next morning. In these latter cases a satisfactory study of most of the finer changes was impossible but observations on organs for blood content, hemorrhage and lipoid substances were usually satisfactory.

In the first sixteen autopsies the following organs were weighed routinely: thyroid, liver, spleen, kidneys, pancreas and gonads. However, since on comparison with normal ratios we found no indication of change following adrenalectomy even in the long-lived animals, the procedure was discontinued and the organ weights were determined only when specially indicated.

Particular attention was paid to the glands of internal secretion, with routine microscopical examination of hypophysis, pineal, thyroid, parathyroid, thymus, pancreas and gonads from paraffin sections stained with hematoxylin and eosin and special stains when indicated.

As controls twenty unoperated cats have been autopsied and histological studies made for comparison. Many of these were animals used in the students' laboratory for various experiments with urethane anesthesia.

From part of these all food was withheld for several days before sacrificing them in order to see how much influence starvation alone would have in the production of the changes to be described later.

RESULTS. The cats survived from a few days to as long as 38 days after the second operation.

Solids in blood and skeletal muscle. Muscle solids were determined in six cats. They ranged from 21.6 to 25.8 per cent, with an average of 24.0 per cent. This did not seem to differ much from normal. However, our data on normal cat's muscle are very scant.

The blood solids in these cats and in three additional ones ranged from 19.4 to 26.3 per cent with an average of 23.1 per cent. This is certainly higher than normal and is not a post-mortem development because determinations were usually made immediately after death and in four instances before death (table 1).

Blood urea. The blood urea was followed in two cats (no. 333 and no. 334) in which there was presumably a *transient adrenal insufficiency* produced by the method described above. Because of the relation to the problem in hand a detailed account of these findings is warranted.

The blood urea was high on the first day in both cats and increased to the high point (108 and 126 mgm. per 100 cc.) on the fourth day. On the eighth day it was still 96.5 and 94.0 mgm. On the tenth day it fell to 75.0 and 72.0 mgm. in cats 333 and 334 respectively. On the twenty-sixth day it was 73.6 mgm. in cat 334. On the thirty-ninth day it was 70 mgm. in cat 334.

The urea was still above normal on the forty-sixth day, being 72 and 60 mgm. respectively. It returned to normal some time before the seventy-third day as it was then 54.7 and 47.0 mgm. respectively.

The animals following the second operation, behaved much as do animals with both adrenals removed except that muscular weakness did not develop to an appreciable degree. Both remained quiet and took little interest in their surroundings. Cat 334 was troubled with diarrhea and appeared in poorer condition than cat 333.

At eighty days the left adrenal was removed from each animal. Cat 333 died twenty hours after the operation with symptoms typical of acute insufficiency. Cat 334 died on the fifth day after the operation. The kidneys were found somewhat congested.

We succeeded in producing a transient insufficiency in a Belgian hare by completely tying off the adrenals in a two-stage operation, forty days apart. Following the second operation, the animal began to eat toward the end of the first twenty-four hours. The feces were semi-fluid. On the third day he was eating very little. On the fourth day he appeared so weak that we prophesied death within a few hours. The blood urea was 108 mgm. per 100 cc. at this stage. Twenty-four hours later the animal had nearly

TABLE 1

Blood changes in adrenalectomized cats

CAT	BLOOD UREA MGM.		BLOOD SUGAR MGM.		BLOOD CALCIUM	BLOOD PHOSPHORUS	BLOOD SOLIDS
	Day	Per 100 cc.	Day	Per 100 cc.	mgm. 100 cc.	mgm. 100 cc.	
G ♂ 3.37 kgm.	Before	20.4					
	1 after	24.2					
2 days between operations	2 after	56.9					
	3 after	53.5					
	4 after	55.0	8	94	10.6	5.7	10th day, 22.2%
	5 after	66.9	11	78	12.2	8.4	At death, 22.4%
Survived 16 days	7 after	60.3	14	68	10.0	5.0	
	8 after	65.4	At death	58	12.4	14.0	Red muscle, solids, 24.8%
	At death	116.0					
H ♂ 3.65 kgm.	Before	20.18					
	1 after	23.6					
2	2 after	44.8					
	3 after	44.6					
Survived 13 days	4 after	55.6					
	5 after	58.7	8	76	9.2	7.6	10th day, 21.0%
	7 after	76.7					
	8 after	60.6	11	80	8.7	8.8	
	At death	177.0	Death	55	10.0	16.6	
I ♂ 2.69 kgm.	Before	36.4					
	1 after	23.2					
2	2 after	56.9					
Survived 11 days	4 after	76.7	8	82	9.9	6.9	10th day, 22.7%
	7 after	83.1	11	66	9.3	11.4	
	8 after	71.7	Death	73	10.4	30.6	
J ♂ 2.63 kgm.	Before	24.7					
	1 after	51.1					
2	2 after	71.4					
	4 after	95.2					
Survived 11 days	5 after	83.4	8	87	11.5	7.5	10th day, 21.6%
	6 after	47.2	At death	54	11.3	21.4	
	7 after	68.2					

recovered his strength and was eating well. The blood urea had dropped to 66 mgm. Eleven days after the second adrenal operation, the animal

had completely recovered and appeared perfectly normal. The blood urea was 40.5 mgm. This animal died about sixteen months later after being exercised in the treadmill.

In these experiments the increased blood urea seemed to parallel adrenal insufficiency and indicated a possible test for this condition.

The blood urea was followed in forty-seven cats with *complete adrenal insufficiency*. In twenty-five of these the determinations were made daily. In the others, determinations were made at intervals of a few days. Typical changes are shown in table 1.

The blood urea may increase following the removal of one adrenal but it soon returns to normal. After the removal of the second adrenal, the urea usually rises within the first two or three days to about double the normal amount. It remains at this higher level with few exceptions until death. Just before death ordinarily the urea becomes much higher.

Occasionally, the urea reached high figures a few days after the second operation and then gradually returned to lower values, which were still above normal.

Attempts have been made to modify the blood urea in some of the adrenal-insufficient animals.

The injection of fluid seems to reduce the blood urea to some extent. Thus, one cat was receiving 35 cc. of fluid subcutaneously every day through the sixth day. The blood urea had risen to 222 mgm. The fluid was increased to 75 cc. daily. The urea dropped to 141 mgm. on the seventh day and to 56 mgm. on the eighth day.

However, another cat was treated in exactly the same way without so marked a change in blood urea, it being 80 mgm. on the sixth day, 117 mgm. on the seventh day (fluid increased to 75 cc. daily) and 74 mgm. on the eighth day. The urea would probably have gone higher in this cat if it had not been for the injection of fluid.

It is known that food may increase the blood urea in normal cats to some extent. We accidentally found that liver will increase the blood urea greatly in some adrenalectomized cats. The urea may not only rise but the symptoms may become quite severe from the intake of too much food and the life of the animal may be shortened. The following instances serve as an illustration.

On the sixth day after removal of the second adrenal three cats, which were in good condition and eating well up to that time, were each fed a large amount of fresh liver. Previously, these cats had not been given liver. In sixteen hours one cat was in distress breathing fifty labored respirations per minute (28 normal). This cat died twenty-six hours after feeding the liver. The blood urea was 295 mgm. per 100 cc. It had been high (82.5 mgm.) two days before the liver feeding.

The second cat (yy) became, within three days, so weak that it could

scarcely walk without falling. The blood urea rose to 120 mgm. (It was 48 mgm. two days before the liver feeding.) Five days after the liver feeding the cat had become stronger and had recovered his appetite. The blood urea had then dropped to 38.7 mgm. This cat lived fifteen days after adrenalectomy with a blood urea of only 69.5 mgm. at death. The day before death the blood urea was 34.4 mgm.

The third cat (B. W.) never recovered its appetite after the feeding of the liver although it lived twelve days longer.

Another cat at a different time (weight, 4.02 kgm.) was fed only 22 grams of liver on the eleventh day after removing the second adrenal. Up to this time the cat was in good condition. He died in less than sixteen hours.

Blood sugar. The blood sugar begins to fall within a few days after the operation. During the last days of life it falls markedly (table 1) until it approaches convulsion levels in some instances. In some of our animals it has reached as low as 49 mgm. per 100 cc.

In the two cats (333 and 334) in which a transient adrenal insufficiency was produced by the method already described, the blood sugar was respectively: on the fifth day, 76 and 87 mgm.; on the eighth day, 100 and 85 mgm.; on the tenth day, 80 and 72 mgm. and on the twelfth day, 80 and 80 mgm.

A lower blood sugar, therefore, seems to be present in adrenal insufficiency. It is uncertain whether the terminal picture in adrenalectomized cats is partly due to the low sugar.

Calcium. The blood calcium was somewhat above normal, ranging from 8.4 to 13.5 mgm. per 100 cc., with an average of 10.35 mgm. for thirteen cats (see also table 1). These slightly higher values probably have no special significance.

Uric acid. Substances reacting with the uric acid reagent ranged from 6 to 15 mgm. per 100 cc. with an average of 10.2 mgm. (4 cats). Values such as these are consistent with impairment of kidney function.

Creatinin. The blood creatinin ranged from 2.5 to 3.4 mgm. per 100 cc. with an average of 2.95 mgm. (4 cats). Disturbance of kidney function may account for this small creatinin excess.

Phosphorus. In the terminal stage the blood phosphorus present as acid soluble phosphates was considerably higher than normal, ranging from 13.3 to 30.6 mgm. with an average of 18.92 mgm. per 100 cc. (9 cats). This increase began shortly before death (table 1). The normal blood phosphorus as determined in four cats ranged from 6.3 to 11.0 mgm. per 100 cc. with an average of 8.1 mgm. Excess phosphates in blood is further evidence of impairment of kidney function.

Anatomical findings. This series of observations includes thirty-five cats in which double adrenalectomy had been performed and in which the animals had been kept alive for periods varying from two to thirty-eight

days. Only one animal survived a shorter period than two days after the second operation. The ages of the animals were not ascertainable but the largest number consisted of young adults all of which were males except four; one of the females was pregnant.

The following changes have occurred with a striking degree of regularity:

1. *Discoloration of the gums.* A purplish discoloration of the gingival margins was present in greater or lesser degree in the great majority of the animals. In some instances it was associated with submucosal hemorrhage.

2. *Hemorrhages in the thymus.* These were not in every case apparent grossly. When visible they appeared as punctate to pinhead sized spots under the capsule and on section were uniformly distributed throughout the gland. Microscopically, the smaller vessels were usually distended with blood, and here and there the tissue was diffusely infiltrated with extravasated blood. Both cortex and medulla were involved. Such hemorrhages occurred in only one of the control animals and this was a cat which died of infection (K) following a series of subcutaneous injections.

3. *General hyperemia.* Hyperemia occurred with considerably frequency in the thymus, hypophysis, liver, kidney and spleen. It was more often absent than present in the pancreas. In the hypophysis it consisted of a dilatation of the capillaries more particularly of the anterior lobe. The liver showed a general dilatation of its sinusoids, associated in some instances with the typical picture of chronic passive congestion. A moderate grade of atrophy of the liver cords particularly in the central portions of the lobules was frequently present. In the kidney the hyperemia involved the capillary tufts of the glomeruli but was not, however, associated with extravasation of blood into the capsule of Bowman in any case.

4. *Changes in the kidney.* These were the most constant as well as the most striking. They consisted essentially of an increase in the lipoid content of the cortex. On the surface the organ showed nothing unusual except a moderate dilatation of the stellate veins. On section the pyramids were congested and thus distinctly demarcated from the cortex which appeared pale, slightly swollen as a rule and grayish-yellow in color In some of the animals that had survived for a longer period the renal cortex had a golden yellow color.

Microscopically, in hematoxylin and eosin sections, aside from accidental findings, the picture was quite characteristic. There was usually high grade general hyperemia involving chiefly the cortex. Swelling and vacuolization of the epithelium of the tubuli contorti were constant. The vacuoles tended to be smaller and fewer in number in the short-lived animals while in those surviving for a longer period they were large and so closely packed together that the outlines of the epithelial cells were greatly

distorted. In frozen sections stained with Sudan III large quantities of lipoid substances were demonstrated in the form of large and small droplets in the tubular epithelium, corresponding in location to the vacuoles visible

TABLE 2

Kidney changes—operated animals

CAT NUMBER	NO. DAYS SURVIVED	LIPOID CONTENT	HYPEREMIA
OY	36	++++	++++
G	16	+++	++
H	13	+++	+++
I	11	+++	+++
J	11	+++	++
M	18	+++	++++
N	8	+	+++
O	7	+	++
P	22	++++	+++
Q	3	+++	++
R	9	+	+
S	4	+	++
T	4	++	++++
U	38	++++	0
V	3	+++	+
W	14	++	0
X	9	+	++
Y	5	+	++
Z	14	+++	++
AA	27	+++	0
AB	12	?	+
AC	2	+++	+++
AE	26	++++	++
AG	11	+++	0
AH	13	S	++
AI	2	+++	+++
AJ	1	++	++
AK	6	S	0
AL	9	+++	0
AM	11	++	0
AO	3	+++	+++
AP	3	+	0
AQ	2	+	++
AR	7	+++	0
AS	3	+++	++

S = Scant.

in paraffine section. Reference to tables 2 and 3 will give a rough estimate of the quantity of lipoid staining with Sudan III. (See also figs. 1 and 2.) In similar sections stained with Nile blue sulphate, the larger portion of the lipoid was colored light red or bluish-red. The remainder showed

various intensities of blue. While the greater accumulation of lipoid occurred in the tubuli contorti, an increase was frequently observed also in the loops of Henle and occasionally in the epithelium of the collecting tubules; in some instances fatty casts were found in the lumina of the latter.

On the basis of these staining reactions an attempt was made to ascertain as nearly as possible, the nature of the lipoids. While it is now generally accepted that staining reactions can, at best, serve only as a rough index of the chemical nature of lipoid substances, they can, nevertheless, furnish

TABLE 3

Kidney changes—control animals

CAT NUMBER	LIPOID CONTENT	HYPER-EMIA	REMARKS
F_1	S	+++	Female with 6-day old kittens
F_2	S	+++	Female—pregnant
F_3	S	+	Female—urethane anesthesia
F_4	++	++	Female—pregnant—urethane
I	S	0	One adrenal removed—starved 5 days
II	S	0	Urethane anesthesia
III	++	0	Urethane anesthesia
IV	+	0	Urethane anesthesia
V	+	0	Urethane anesthesia
VI	++	0	Urethane anesthesia
VII	+	0	Urethane anesthesia
VIII	+	0	Urethane anesthesia
IX	++	0	Urethane anesthesia
X	S	0	Urethane anesthesia
XI	+	0	Denervated liver—urethane
XII	+	0	Urethane anesthesia
XIII	S	0	Denervated liver—urethane
XIV	S	+	Starved 4 days—urethane
XV	+++	+++	Died of infection following subcutaneous injections
XVI	S	++	Urethane anesthesia

S = scant. 0 = none.

a clue for their identification. The major portion of these globules was stained orange or orange-red by Sudan III and red or bluish-red by Nile blue sulphate, indicating the presence of cholesterine-ester and cholesterine-fatty acid mixtures (Mallory and Wright, 1924). A smaller portion was blackened by osmic acid, suggesting a considerable neutral fat fraction.

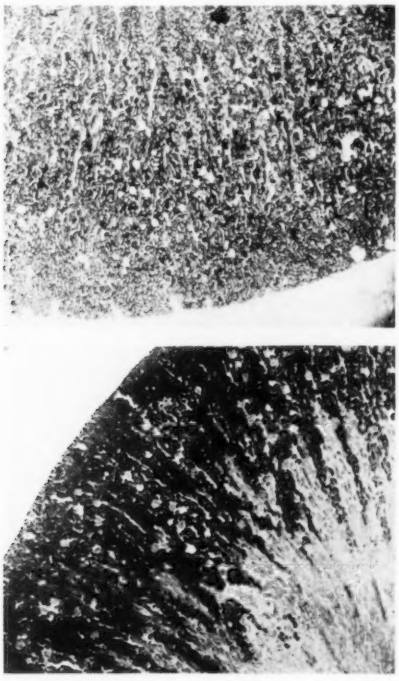
A more detailed description of the anatomical changes will be published at a later date.

DISCUSSION. After our work was nearly completed results having a bearing upon our problem were published from three other laboratories.

Lucas found concentration of blood solids and an increase in blood phosphorus and urea.

Banting and Gairns described hyperemia of the kidney with swelling of the tubules. They also found albuminous casts and blood cells in the tubules.

Rogoff and Stewart found that the non-protein nitrogen of the blood rose



(Upper) Fig. 1. Section through the cortex of a normal kidney stained with Sudan III showing a normal amount (+) of lipoid. Low power magnification.

(Lower) Fig. 2. Section through the kidney cortex of an adrenalectomized animal (OY) showing a large quantity (++++) of stainable lipoid. Sudan III stain.

markedly when the characteristic symptoms, especially anorexia, developed. The increase in urea nitrogen was the great factor in this rise. They also describe an increase in the blood solids.

Swingle found an increase in blood phosphorus and a lowering of the blood sugar. He also found albumin in the urine in animals showing marked symptoms, but was unable to find anything but congestion and hemorrhage in the kidneys.

SUMMARY

Blood changes have been followed in adrenalectomized cats, some of which survived more than thirty-five days.

There was an increase in the blood solids.

The blood sugar fell in the later stages, reaching as low as 50 per cent of the normal at death in some individuals.

The calcium, creatinin and uric acid in the blood were slightly above normal.

The acid soluble phosphates of the blood were considerably higher than normal in the later stages.

The blood urea increased and this increase to some extent ran parallel with the loss of appetite and other symptoms. The ingestion of liver caused a marked increase in blood urea and an exacerbation of the symptoms.

The anatomical changes which appeared rather constantly were: purplish discoloration of the gums, hemorrhages in the thymus, general hyperemia of the internal organs and an accumulation of large quantities of lipoid substances in the tubuli contorti.

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